Afawork IDN/1618

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United States Department Of Commerce
United States Patent and Trademark Office
Commissioner for Patents
P.O. Box 1450
Alexandria Virginia 22313-1450

7 ,February, 2006

Dear Sirs,

referring to your messages (application n° 09/787,312 and following), and according to rules established by United States Patent and Trademark Office, we send you our observations about the notice of rejected claims .

Our remarks follow the numbering employed by Patent Examiner Mr. Michael G. Hartley.

Claim rejections 35 USC - 101 and 112

On page 3 lines 8-10 of our application is very well established that the R (dextrorotatory) form of Iopamidol is less toxic of the S (levorotatory) form of the Iopamidol.

Actually (see United States Pharmacopoeia) the only accepted Iopamidol must contain at least 95% of S form.

On the international market only the formulation that contains at least 95% of S form is contemplated.

Our product is therefore completely new.

We remind you that the subject of our patent are the advantages of the dextrorotatory forms of non-ionic contrast media which show at least one optically active center.

Up till now, no dextrorotatory form of contrast media is on the market and no request of introduction on the market results has been registered.
According to the Pharmacopoeia, dextrorotatory and levorotatory forms of a drug are two distinct entities and have different pharmaceutical properties.

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On the market there are several drugs optically active (for example: levorotatory form of thyroxin or levorotatory form of dopamine, whose dextrorotatory isomers don't have pharmaceutical properties).

The aim of our patent is that of producing a dextrorotatory form of contrast media which presents the properties of an adequate contrast medium (properties which are bound to the presence of iodine atoms) but, at the same time, does not present much or any affinity with the human receptors (property which is bound to the optical structure of the molecule).

No one doubts the validity of the S form of Iopamidol as a non-ionic iodinated contrast medium. Anyway, side effects are reported in 3-4% of the patients and 0,04% of them are rated as grave or fatal. Considering the large diffusion of non ionic contrast media and their increasing employment we can assert that numerous patients are subject to the effects of chemio-toxicity.

The form R of Iopamidol, object of our patent offers a significant reduction of said risk in order to protect the patient's health.

Moreover, we must consider the profile of the product and its specifics, reported into the following table (United States Pharmacopoeia and European Pharmacopoeia):

Parameter	Specification
Organic Impurities -HPLC	Single unknown: Single known: NMT 0.5% Total impurities: NMT 1.5 %
Heavy metals	NMT 10 p.p.m.
Free aromatic amine	NMT 0.05%
Free iodine	negative
Free iodide	NMT 0.02 %
Particulate matter	≥ 10 μm: NMT 6000 per container ≥ 25 μm: NMT 600 per container
Bacterial endotoxine (LAL)	NMT 0.7 EU/ml
Sterility	Sterile

In the formulation with concentration 370 for 100ml, on the market in the UE, there are about 75g of S Iopamidol.

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Therefore in the bottle are permitted the maximum presence of the following substances:

Organic Impurities Total	mg 1130
Single Known	mg 380
Single Unknown	mg 75
Free aromatic amine	mg 38 ca.
Free iodide	mg 150

Moreover we must not forget, as asserted by the literature, that about the 1% of S Iopamidol is bound to the plasmatic proteins, which happens to reach in this concentration the amount of 750 mg.

We must not think that the injectable formulation of S Iopamidol contains 100% of pure raw material. The product on the market can contain and contains, beside the active principle, many other related products that, in the case of contrast media, reach the amount of several dozens of milligrams directly injected in the bloodstream.

If it's probably true that the S Iopamidol isn't affected by biotransformation, it's certainly true and confirmed by the pharmacopoeias that the S Iopamidol contains a high number of admitted impurities.

Because of their particular biological importance, we must remember the free aromatic amines and the free iodide, because potentially able to bind to the human receptor.
It appears clear that the impurities with optically active center must have a levorotatory form (S), to mantain an higher affinity with the human biologic receptors.
We must remember:

I) Cardiac B1 receptors have a specific affinity to the levorotatory molecules (levo atenolol)
II) The active form of the thyroid hormon is the sodic levo-thyroxin (S) which is administred in micrograms.
III) The receptors of inflammation have an high affinity for the form levorotatory (S) of some pharmaceutical molecule (S Ketoprofen, S Ibuprofen, etc.).
IV) The levorotatory form (S) of Warfarin, strong anticoagulant, have an higher affinity to the receptors of coagulation than the form dextrorotatory (R).
V) The cerebral receptors have a specific affinity for the levorotatory (S) form of dopamine and other pharmaceutical substances.

If we consider the side effects that are reported on the SPC of the formulations on the market of S Iopamidol, we'll see that they are of cardiac, thyroid, emathological, neurological and anaphylactic nature.

All these side effects, as confirmed by many researches on the matter, are not related to the hydrophilic property or to physic and chemical properties of the formulation but are related, probably, to the molecular structure that, binding to the human receptor starts a chain reactions that provide immediate and retarded side effects which can be light, grave or even fatal.

Therefore we confirm the validity of the form S of Iopamidol, but, according to what said, we can't assert that the two isomeric forms have the same pharmacologic characteristics.

It's not by chance that the pharmacopoeias order separate studies for all the isomers which have pharmacologic activities, and never accept an identity of their biologic properties.

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The R form of Iopamidol differs deeply from S form.

For istance in a patient affected with hypothyroidism it is different to administer 100 mcg of levo–thyroxin (S form of thyroxin) or100 mcg of dextro-thyroxin (R form of thyroxin)

Claim Rejections 35 USC -102 and 103

1)The US patent n° 4001323 **(annex 1)** asserts:

a) "They are the first non ionic 2,4,6, triiodobenzenederivates which are readily soluble in water, but free from contaminating isomers", in column 1, lines 53-55, referring implicitly to the absence of the contaminating isomer R Iopamidol.

b) in the related examples, column 2, lines 14-34, R Iopamidol is missing.

c) no data are provided to support the assertions in column 4, lines 47-56.

d) column 4 lines 46-56 "the compound of invention which are optically active and particulary the L enantiomorphs [...] are preferred. The D enanthiomorphs of the compound of the invention have not been found to offer advantages over the L-enantiomorphs and none can be expected"

e) the solubility of the S form of Iopamidol is said to be superior to the solubility of the RS form of Iopamidol. In table 1, column 3, the solubility of the form RS at 20° 40° is specified to be 30g/100ml, while at 60° the solubility is specified to be just 32 g.

In 1980 the same authors of the patent published a work **(annex 2)**in which they asserted (page 303, line 13 of the table) that the solubility at 25° is just 16 g per litre. In the graphic at page 307 this data is clearly confirmed at 20° 40° too.

Then, which is the solubility of the form RS of Iopamidol? Does the same molecule have different solubilities?

We would like to stress out that the authors of the patent and of the following publications are the same, Felder and Pitre.

In another work, the same authors, assert that the solubility depends on the crystalline form (page 137, point 3.3.2.) **(annex 3)** and not on the isomeric form. Moreover, the pentahydrate form shows, in picture 8, page 139, almost the same trend of the graphic provided at page 307 of the previously quoted work.

Shortly, the authors assert:

- Firstly, in the patent, that the RS form of Iopamidol has a solubility of 30g/100ml;
- Secondly, in a previous work, that said solubility becomes of 16g/100ml;
- Finally, that the RS form of Iopamidol has the same solubility of the S form of Iopamidol pentahydrate.

We can infer that, were these data true, it would be a peculiar behaviour to be observed in a molecule.

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2) Neither Lorenzini (patent 6875887) **(annex 15)** nor Benanni (patent 5609851) **(annex 16)** have proposed a non-ionic contrast medium based on the dextrorotatory form and which presents an higher tollerability.

The authors didn't even reveal the superior characteristics of the dextrorotatory form compared to the levorotatory form in terms of toxicity, actually considering them to be identical.

Moreover no one hypotized that the side effects of non-ionic contrast media are bound to the property of bounding themselves as contrast media or impurities to the human receptors.

Once again, no one remembered that the human receptors have a peculiar affinity for the levorotary forms, while it is highly unlikely a bond with the dextrorotatory forms.

Therefore the dextrorotatory forms, not binding to the receptor are not able to produce side effects that, in the case of contrast media, happen to be grave and often deadly.

Lastly, we'd like to remind you that Lorenzini's application have a priority data on 26 february 1999 while our priority data is 16 september 1998 (also international filing date and international publication date confirm our consideration)**(annex 13).** Therefore, it would have been Lorenzini the one to use already well-known information. And this notwithstanding he even obtained the patent .

In addition to this incoherence, in Lorenzini's patent there are references to "salt of the Iopamidol". We'd like to remind you that it is chemically impossible to obtain a salt from a non-ionic product. Only ionic compounds can produce salts.

No author seems to have studied the differences occurring between the form S and the form R of Iopamidol. There's no trace of a similar study in the documents provided by the Patent Office.
It is a bit careless to try to foresee the behaviour of a drug through assertion as "no difference nor advantages have to been expected between S and R Iopamidol".
This assertion was made into 1975, when very little was known about chemio-toxicity and biological impact in reception. Anyway, there are no published scientific works supporting this assertion.

The form R of Iopamidol offers a significant reduction of said risk in order to protect the patient's health.

Moreover, we want to present you with the conclusion of several researches published in the last years which confirm the origin of many side effects in the bond between receptor and contrast medium and/or its related substances:

A) Feltrin et alt "Fondamenti sui mezzi di contrasto iodati e reazioni avverse", La radiologia Medica, vol. 107 8-31, 2004 (annex 7)

The author supposes the possibility of an hydrogen bond between the contrast medium and peptide or polypeptides (see page 11 and, fig 5 at page 5). We remind you that all the amino acids in human polypeptide chains are all of levorotatory form.

B) SK Morcos "Contrast media-induced nephrotoxicity" The British Journal of radiology vol 71 issue 844 pag 357-365, 1998 (annex 8)

The author supposes a fundamental role of Endothelin and adenosine in mediating some side effect of contrast media. The release would presumably happen through receptors. To confirm his hypothesis the author affirms that "the prophylactic administration of calcium channel blockers and adenosine antagonists such as theophylline may also offer some protective effect."

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C) Y-XJ Wang et alt "Radiographic contrast media induced nephropathy:experimental observation and the protective effect of calcium channel blocker "The British Journal of radiology vol 74 (2001) 1103-1108 (annex 9)

The authors reach the same conclusions as Morcos but they offer a larger spectrum of drugs which oppose the receptors, thus preventing nephropathy brought by contrast media.

D) Blann et alt "Changes in endothelial,leucocyte and platelet markers following contrast medium injection during angiography in patients with peripheral artery disease" The British Journal of radiology vol 74 (2001) 811-817 (annex 10)

The results convalidate the hypothesis of an impact on the receptors, with an heavy action on the principal markers.

E) Laffan et alt "A comparison between the platelet activating properties of different contrast media used in radiology and MRI" The British Journal of radiology vol 740 (1997) 798-804 (annex 11)

The authors demonstrate the impact of different contrast media on platelet activating properties. Moreover is confirmed the increase of bound fibrinogen.

F) SK Morcos "Effect of radiographic contrast media on the lung The British Journal of radiology vol 76 (2003) 290-295 (annex 12)

The authors assert in the abstract: "Pre-treatment with corticosteroids or antihistamine does not appear to prevent radiographic contrast media induced bronchospasm ,but the administration of b2 adrenergic agonist can abolish this adverse side effect."

We expertised only a little portion of the studies which show the side effect of contrast media coming from a mechanism of stimulation or inhibition of receptors.

A solution to the side effects was the employment of bigger molecules as dimeric non-ionic contrast media (Iopamidol is a monomer), thus provoking a stechiometric obstruction.

Our solution consists, more simply, in reducing the receptorial affinity, thus making the molecule unable to bind through the employment of the biologically inactive isomer(R-form).

Non one has previously reached these conclusions.

On 04 August 2004 the European Patent Office has granted the patent after a throughout examination, including the publication quoted by the patent examiner. (annex 14)

Bracco Imaging company had filed a notice of opposition against the patent and we are waiting for the European Patent Office decision. Copy of the opposition cited publication are included in our annex.

Lastly, we look forward for your reply and we confirm our availability to any further investigation or explanation of the matter.

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Sincerely,

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1)US patent n° 4001323

2)Pitrè and Felder "Development,Chemistry,and Physical Properties of Iopamidol and its Analogues" Investigative Radiology November-Dicember 1980 suppl. Vol 15 N° 6

3) Pitrè , Felder and alt " Analytical Profiles of Drug substance Vol 17 Iopamidol"

4) De Haen et alt of Milano Research Centre,Bracco Spa, Italy "Neurotolerability of non ionic X- ray contrast media. The role of chemotoxicity." Invest. Radiol 1996 Jun;31(6)338-44.

5) Grandi and Pitre, Bracco Industria Chimica, "Fast Atom Bombardment Mass Spectrometry of By-products in the Iopamidol Synthesis", Biomedical Mass Spectrometry, vol 10, pag. 17-23, N° 1 1983

6) BIAM Iopamidol

7) Feltrin et alt "Fondamenti sui mezzi di contrasto iodati e reazioni avverse", La radiologia Medica, vol. 107 8-31,2004

8) SK Morcos "Contrast media-induced nephrotoxicity" The british Journal of radiology vol 71 issue 844 pag 357-365 1998

9) Y-XJ Wang et alt "Radiographic contrast media induced nephropathy:experimental observation and the protective effect of calcium channel blocker "The British Journal of radiology vol 74 (2001) 1103-1108

10) Blann et alt "Changes in endothelial,leucocyte and platelet markers following contrast medium injection during angiography in patients with peripheral artery disease" The British Journal of radiology vol 74 (2001) 811-817

11) Laffan et alt "A comparison between the platelet activating properties of different contrast media used in radiology and MRI" The British Journal of radiology vol 740 (21997) 798-804

12) SK Morcos "Effect of radiographic contrast media on the lung The British Journal of radiology vol 76 (2003) 290-295

13)Patent Application n° 09/787,312 / international publication WO 00/50385 and 00/15266

14)European Patent N° 1113823

15)USP 6875887

16)USP 5609851

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Development, Chemistry, and Physical Properties of Iopamidol and Its Analogues

D. PITRÈ, PHD, AND E. FELDER, PHD

A series of 5-hydroxyacylamino-2,4,6-triiodo-N,N'-hydroxyalkyl-isophtalamides was prepared in order to study the structural requirements for high water solubility and low toxicity. Among the amides substituted by 2-hydroxyethyl; 2,3-dihydroxypropyl; 1,3-dihydroxypropyl-; and 1,1-dihydroxymethyl-2-oxyethyl- groups, the highest water solubility was obtained with the 1,3-dihydroxypropyl- group. Neither optical resolution of the 2,3-dihydroxypropyl moiety nor amide formation with two different amines improved the water solubility. The optimal solubility was obtained with S-5-α-hydroxypropionylamino-2,4,6-triiodoisophtalic acid di-(1,3-dihydroxy-2-propylamide), Iopamidol. Iopamidol forms anhydrous and monohydrate crystals, characterized by differential thermal analyses, x-ray diffraction patterns, and solubility patterns. A method for enzymatic assay of the optical purity of Iopamidol with lactodehydrogenase is described as well as partition coefficient, ionization constant of the oxyacylamido-group, critical micelle formation, surface tension, and osmolarity.

Key words: Iopamidol, nonionic water-soluble contrast media, synthesis.

TO DEVELOP new, nonionic, water-soluble contrast media, we used structural elements used in established ionic contrast media for urography, such as diatrizoic, iodamic, iothalamic, and acetrizoic acids. Thus, we retained in our design the triiodinated benzene ring as the radiopaque moiety containing stable carbon–iodine bonds. Further, we substituted the benzene ring with substituents that should presumably induce low toxicity and high water solubility. In this article we shall discuss compounds based on the 5-amino-2,4,6-triiodoisophtalic acid.

The series of compounds was developed according to the scheme shown in Table 1, which shows the synthesis of the hydroxyalkylamides of 5-hydroxyacylamino-2,4,6-triiodoisophtalic acid.[1,2] We chose the amido and hydroxyacyl groups to avoid the formation of restricted rotational isomers and to retain a high iodine content in the molecule.

Experimental results, reported in Table 2, show that the highest solubility in water was obtained with 1,3-dihydroxyisopropyl substituted amide and that optical activity of compounds containing the 2,3-dihydroxypropyl group, if any, had no important effect on solubility. Furthermore, asymmetric amides (ie, those with two different amide substituents) were found to be generally less water soluble than the symmetric ones.

The structural contribution of the hydroxyacyl group becomes important in case of the L-lactoyl group, which made the molecule remarkably soluble, whereas the corresponding D,L-derivative was much less soluble.

Radiopacity, water solubility, osmolarity and viscosity, stability of aqueous solutions, systemic and local toxicity, and pharmacodynamic and pharmacokinetic tests determined that (L) 3-hydroxypropionylamino-2,4,6-triiodoisophtalyl-bis(1,3-dihydroxypropyl-amide) (Iopamidol) (Fig. 1) was the best compound of the series.[3,4]

Since the steric configuration proved important for the water solubility, a method for quantitative determination of optical purity was developed (Table 3). It is based on catalytic deiodination, hydrolyzation in alkaline

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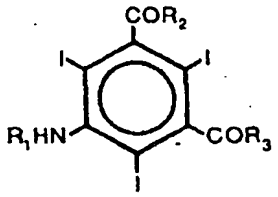
0020-9996/80/1100/S301/$00.90 © J. B. Lippincott Company

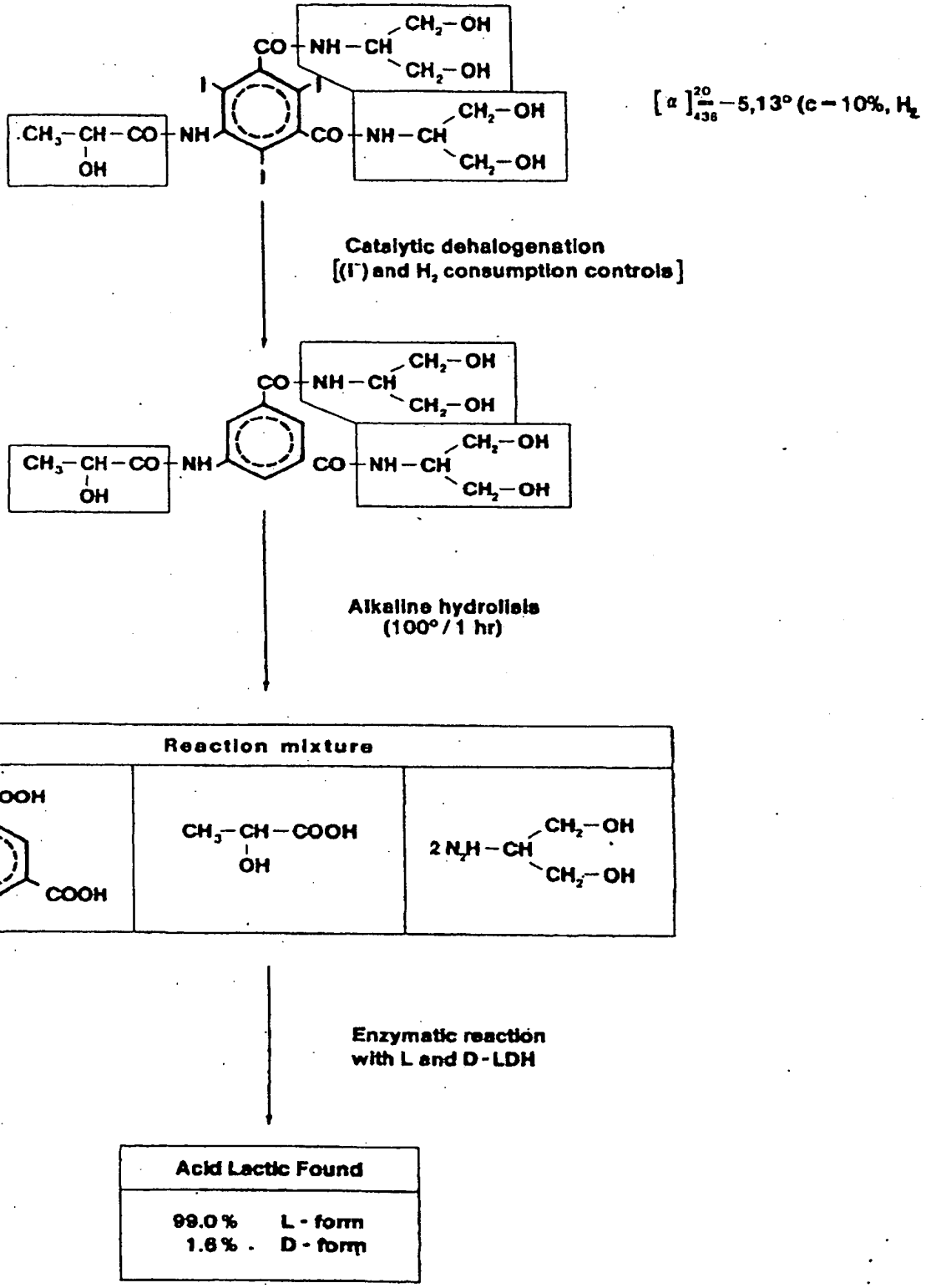
TABLE 1. Scheme of the Synthesis of Iopamidol and Its Analogues

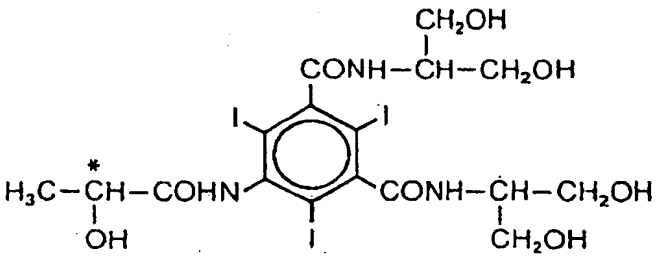
TABLE 2. Water Solubility of Iopamidol and its Analogues



COMPD. NO.	R_1	R_2	R_3	$[\alpha]^{20}_{436}$ (H_2O)	g/100 ml H_2O at 25°
1	$HOCH_2CO-$	$-NHCH_2CH_2OH$	$-NHCH_2CH_2OH$	—	INS.
2	$HOCH_2CO-$	(RS)-$NHCH_2CHOHCH_2OH$	(RS)-$NHCH_2CHOHCH_2OH$	—	1.8
3	$HOCH_2CO-$	$-NHCH(CH_2OH)_2$	$-NHCH(CH_2OH)_2$	—	4.2
4	(S) $CH_3CHOHCO-$	$-NHCH_2CH_2OH$	$-NHCH_2CH_2OH$	—	INS.
5	(S) $CH_3CHOHCO-$	$-NHCH(CH_2OH)_2$	$-NHCH(CH_2OH)_2$	−5.13°	90
6	(S) $CH_3CHOHCO-$	(RS)-$NHCH_2CHOHCH_2OH$	(RS)-$NHCH_2CHOHCH_2OH$	−4.91°	13
7	(S) $CH_3CHOHCO-$	(RS)-$NHCH_2CHOHCH_2OH$	(S)-$NHCH_2CHOHCH_2OH$	−10.19°	12
8	(S) $CH_3CHOHCO-$	(S)-$NHCH_2CHOHCH_2OH$	(S)-$NHCH_2CHOHCH_2OH$	−14.82°	12
9	(S) $CH_3CHOHCO-$	$-NHCH_2CH_2OH$	(RS)-$NHCH_2CHOHCH_2OH$	−4.95°	5
10	(S) $CH_3CHOHCO-$	$-NHCH_2CH_2OH$	$-NHCH(CH_2OH)_2$	—	2
11	(S) $CH_3CHOHCO-$	(RS)-$NHCH_2CHOHCH_2OH$	$-NHCH(CH_2OH)_2$	−4.52°	12
12	(S) $CH_3CHOHCO-$	$-NHC(CH_2OH)_3$	$-NHC(CH_2OH)_3$	−3.40°	2
13	(RS) $CH_3CHOHCO-$	$-NHCH(CH_2OH)_2$	$-NHCH(CH_2OH)_2$	—	16
14	(RS) $CH_3CHOHCO-$	(RS)-$NHCH_2CHOHCH_2OH$	(RS)-$NHCH_2CHOHCH_2OH$	—	12
15	(RS) $CH_3CHOHCO-$	(RS)-$NHCH_2CHOHCH_2OH$	(S)-$NHCH_2CHOHCH_2OH$	−4.92°	12
16	(RS) $CH_3CHOHCO-$	(S)-$NHCH_2CHOHCH_2OH$	(S)-$NHCH_2CHOHCH_2OH$	−9.76°	14
17	(RS) $HOCH_2CHOHCO-$	$-NHCH(CH_2OH)_2$	$-NHCH(CH_2OH)_2$	—	5.5
18	(RS) $CH_3CHOHCHOHCO-$	$-NHCH(CH_2OH)_2$	$-NHCH(CH_2OH)_2$	—	4.5
19	(RS) $CH_3CHOHCHOHCO-$	(RS)-$NHCH_2CHOHCH_2OH$	(RS)-$NHCH_2CHOHCH_2OH$	—	12

TABLE 3. Scheme of the Enzymatic Assay of the Optical Purity of Iopamidol





M.W. 777.29 I=49.00%

IOPAMIDOL

Fig. 1. Structure of iopamidol.

solution *to* 5-aminoisophtalic acid, 2-amino-1,3-propanediol and lactic acid, with no racemization.

Iopamidol occurs in two different crystalline forms, one anhydrous and the other hydrated (Figs. 2, 3) with distinct x-ray powder difractograms and also different elemental cell characteristics (Table 4).

Also, at DTA analysis the two forms differ considerably. As shown in Fig. 4, the anhydrous form reveals a first broad endotherm at 193° and another endotherm with decomposition and iodine liberation at approximately 323°. The hydrated form, instead, shows an endotherm at 127° due to loss of molecular water and another group of endotherms at 240° and 265°, plus a definite exothermal transition in the region of 295°, due to decomposition. The water solubility curves, as a function of temperature, also illustrate the difference between the two forms (Fig. 5). It can be seen that curves are U-shaped, showing the minimum solubility at 23° for the hydrated and at 60° for the anhydrous form. A

TABLE 4. Crystal Data of Iopamidol

Parameters	Anhydrous Form	Hydrated Form
a	11.172 Å	13.611 Å
b	12.403 Å	15.591 Å
c	9.180 Å	12.608 Å
α	92° 48'	66° 25'
β	104° 38'	81° 41'
γ	108° 40'	87° 10'
U	1154.34 Å³	2428.23 Å³
d_o	2.10 g/cu cm	2.12 g/cu cm
d_c	2.237 g/cu cm	2.18 g/cu cm
Z	2	4
Space group	$P\bar{1}$	$P\bar{1}$

The unit cell parameters were determined by Philips PW 1100 computer-controlled four-circles diffractometer using Mo-Kα radiation monochromatized with a graphite crystal.

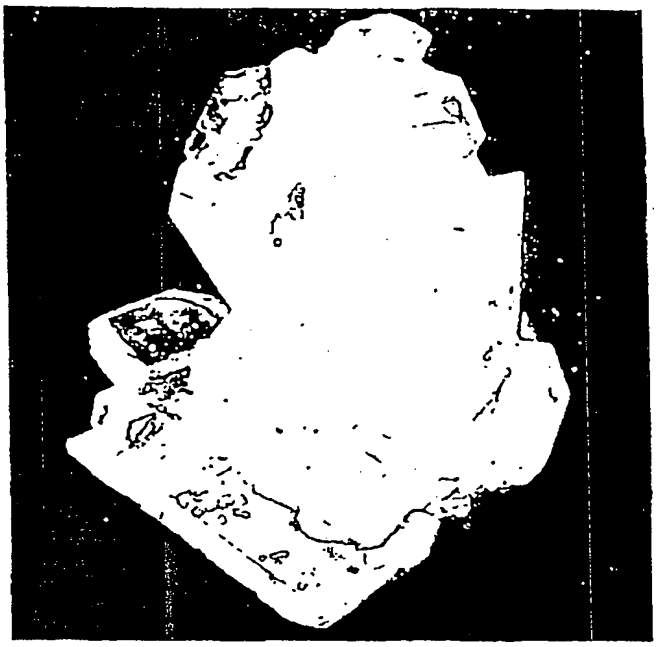
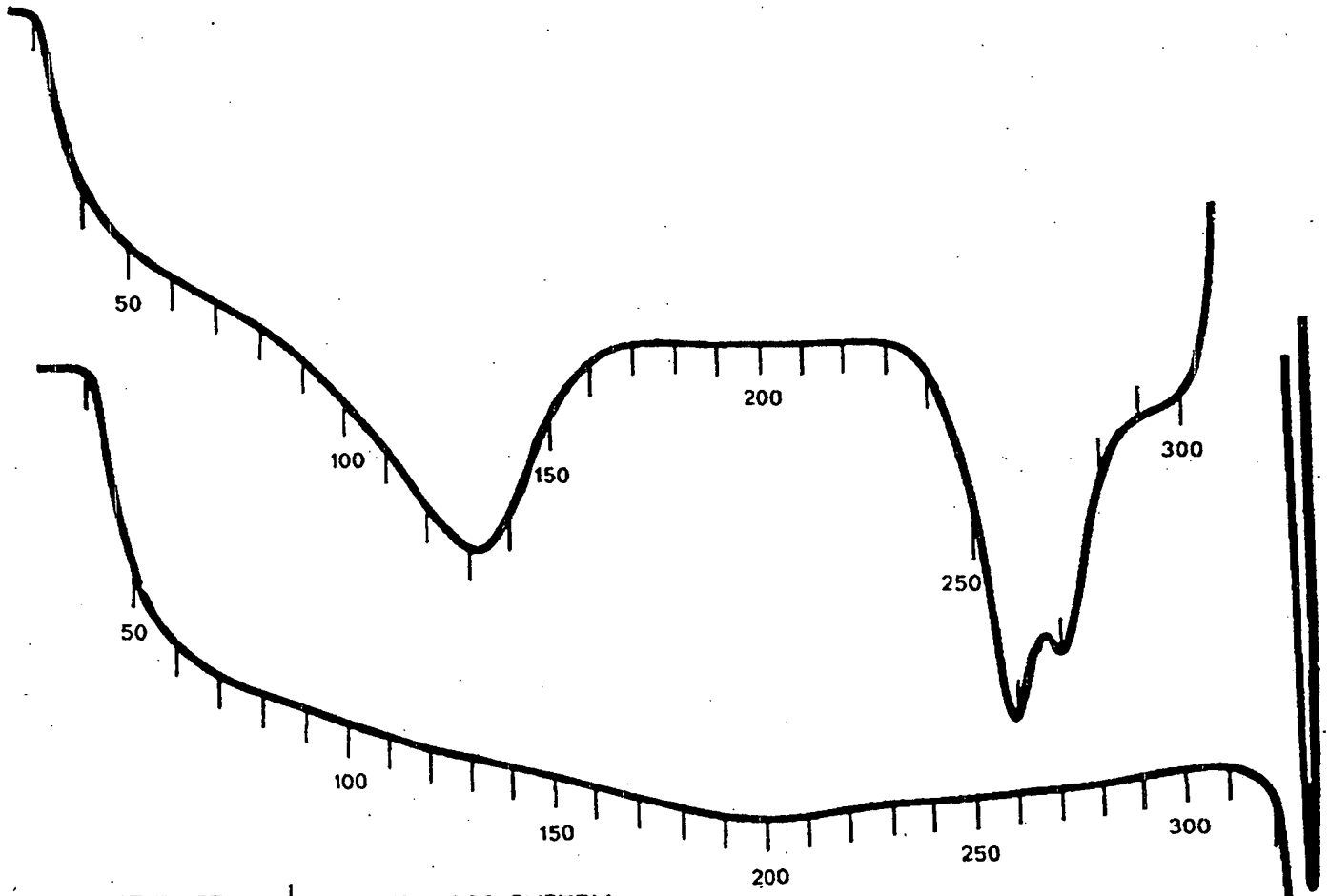


Fig. 2. Anhydrous Iopamidol crystals.

Fig. 3. Hydrated Iopamidol crystals.



METTLER	TA 2000 SYSTEM		
IDENTIFICATION	IOPAMIDOL		
SAMPLE	HYDRATE FORM (upper curve) ANHYDROUS FORM (lower curve)		
PRETREATMENT			
REFERENCE			
CRUCIBLES			
ATMOSPHERE	NITROGEN	AT 20	ml/min
SCAN SPEED		10	°C/min
RANGE		100	µV/fsd
CHART SPEED		10	mm/min
SENSITIVITY $_{In}$		15,2	µV/mW

Fig. 4. DTA curves of the anhydrous and hydrate forms of iopamidol.

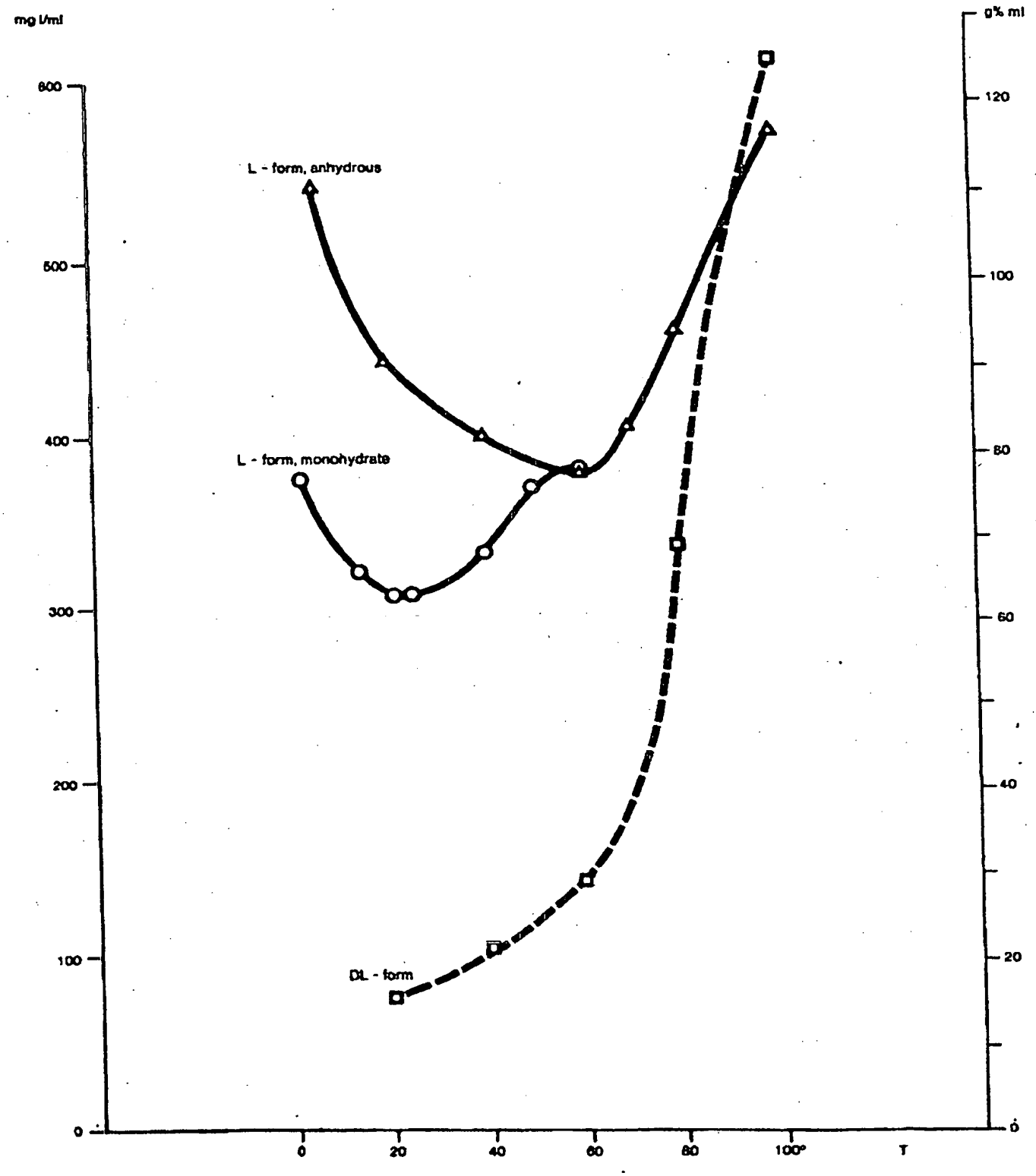
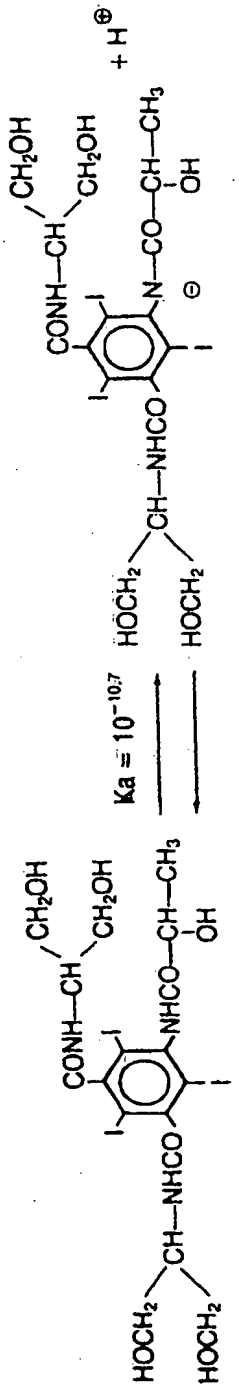


Fig. 5. Solubility of Iopamidol in water.

TABLE 5. Dissociation of Iopamidol



hydrate–anhydrate transition point is in the region of 60°. The presence of hydrophilic groups accounts for low partition coefficients (0.0025 in n-octanol-water and 0.113 in n-butanol-water). Under the same conditions,

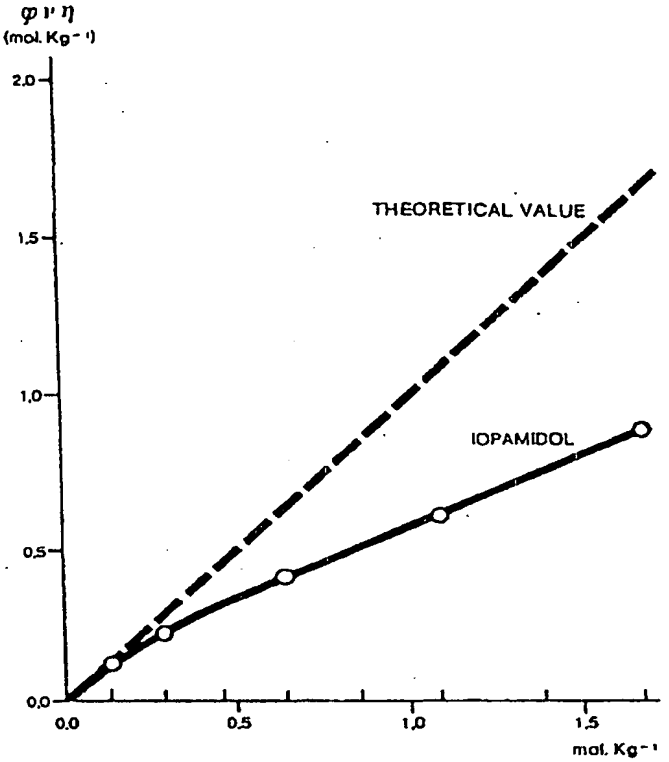


Fig. 6. Osmolality of iopamidol solutions at 37 C.

metrizamide showed partition coefficients between butanol and water of 0.424 and between octanol and water of 0.018.

It is possible to obtain salts of iopamidol with strong alkali in aqueous solution. This is analogous to sulfonamides having a similar dissociation constant. However, on potentiometric titration with NaOH 0.1 N in aqueous solution, a dissociation constant of Ka = $10^{-10.7}$ was found, indicating that at physiologic pH values the dissociation should be insignificant (Table 5).

The osmotic pressures of solutions are shown in Fig. 6. At 370 mg I/ml (corresponding to 0.972 mol/l),

TABLE 6. Dynamic Surface Tension of Aqueous Iopamidol Solutions at 20 C

Iopamidol (mg I/ml)	Surface Tension (dyne/cm)						
	O hr	1/4 hr	1/2 hr	1 hr	2 hr	4 hr	16 hr
0	72.8	—	—	—	—	—	—
50	68.7	67.9	67.3	66.3	64.8	62.5	55.4
100	65.3	63.9	63.0	61.6	59.8	57.5	52.5
200	60.7	58.8	56.9	55.8	54.1	53.1	51.1
300	59.0	56.7	55.1	53.4	52.3	51.8	50.8
400	58.3	54.2	52.9	51.8	51.2	51.1	50.6

TABLE 7. Physicochemical Properties of Aqueous Solutions of Iopamidol

Concentrations		Osmolality	Φ	Viscosity (cP)		Density	
Iodine	Iopamidol	(mol/kg^{-1})	(atm)				
mg/ml	% W/V	37 C	37 C	20 C	37 C	20 C	37 C
400	81.6	0.903	23.0	32.3	12.5	1.44	1.43
370	75.5	0.799	20.3	18.5	8.6	1.41	1.40
350	71.4	0.738	18.8	14.5	7.5	1.39	1.38
300	61.2	0.616	15.7	8.8	4.5	1.34	1.32
200	40.8	0.413	10.5	3.4	2.0	1.22	1.21

iopamidol's osmolarity is 47% lower than that of the equimolar solution of glucose. The surface tension of iopamidol aqueous solutions declines with time, becoming constant within a few hours (Table 6).

The surface tension data are very close to those of uro-angiographic contrast media, sodium and methylglucamine salts of diatrizoic, metrizoic and iothalamic acids (from 64–67 dyne/cm for 0.1 M solutions and 52–62 dyne/cm for 0.5 M solutions). Thus, like the other uro-angiographic contrast media, iopamidol has a weak effect on the surface tension.

Studies of iopamidol solutions, using pH variation, hydrolysis of acyl- and hydroxyalkylamido groups, and deiodination of the molecule as indicators, demonstrated that solutions containing small quantities of buffer and chelating agent are stable enough for heat sterilization (Table 7). After two years of storage at room temperature, the solutions of iopamidol still met the requirements of the Pharmacopeia for current uro-angiographic contrast media.

References

1. Felder E, Pitrè D. U.S. Patent 4,001,323.
2. Felder E, Pitrè D. U.S. Patent 4,139,605.
3. Schmid HW. Untersuchungen der Toxizitaet, der physikalisch-chemischen Eigenschaften und haemolytischen Activitaet von trijodierten Roentgenkontrastmittel-Salzen in Waessrigen Loesungen. Pharm Acta Helv 1971;46:134.
4. Felder E, Pitrè D, Tirone P. Radiopaque contrast media XLIV. Preclinical studies with a new nonionic contrast agent. Farmaco (Sci) 1977;32:836.

Analytical Profiles of Drug Substances

Volume 17

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References

1. INTRODUCTIO

1.1 Foreword

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1.2 History

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1.3 Therapeuti

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1. INTRODUCTION

1.1 Foreword

Iopamidol is an injectable iodinated contrast agent for angiography, excretory urography, and myelography. It belongs to the class of non-ionic triiodinated benzoic acid derivatives with 3 iodine atoms per particle in solution (ratio 3 contrast media).

1.2 History

With the aim of lowering chemotoxicity and improving heat stability of non-ionic molecules a series of hydroxyalkylamides of 5-(α-hydroxyacyl) amino-2, 4,6 triiodoisophthalic acids was synthetized in the Research Laboratories of Bracco Industria Chimica, Milan Italy, in the early 70's.
Shielding the hydrophobic iodine atoms with highly hydrophilic substituents afforded compounds with promising characteristics. Especially the molecule coded B 15000, later to be named Iopamidol, was deemed worthy of preclinical and clinical development and patent protection (1,2,3,4).
Favourable physico-chemical properties, stability to heat sterilization of the injectable solution , and excellent tolerability allowed broad diagnostic indications for Iopamidol such as lumbar and cervical myelography, cerebral angiography, peripheral arteriography and venography, angiocardiography, coronary arteriography, aortography, selective visceral angiography, CT enhancement, digital subtraction angiography, excretory urography and arthrography.
Updated preclinical and clinical experiences were reported during a worldwide Symposium on Iopamidol, held at Fort Lauderdale in early 1983 (5).
Iopamidol was introduced on the Italian market in September 1981 and soon afterwards in other European countries, whereas U.S.introduction followed at the beginning of 1986.

1.3 Therapeutic category

Diagnostic aid

2. DESCRIPTION

2.1 Nomenclature

2.1.1 Chemical Names

1,3-Benzenedicarboxamide-N,N'-bis [2-hydroxy-1-(hydroxymethyl) ethyl]-5-[2-hydroxy-1-oxopropyl)-amino]-2,4,6-triiodo-,(S)-

N,N'bis(1,3-dihydroxy-2-propyl)-5-L-lactoylamino-2,4,6-triiodoisophthalamide.

(S)-N,N'-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-2,4,6-triiodo-5-lactamidoisophthalamide

CAS : 60166-93-0

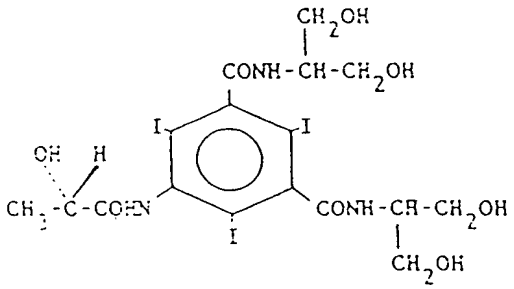
2.1.2 Generic Name

Iopamidol (USAN, INN, BAN)

2.1.3 Trade Names

Iopamiro	(Bracco)
Isovue	(Squibb)
Niopam	(Merck, UK)
Solutrast	(Byk Gulden)
Iopamiron	(Schering)

2.2 Formula, Molecular weight and Iodine Content



$C_{17}H_{22}I_3N_3O_8$ Mol.wt = 777.1

Organically bound iodine : 49.00%

2.3 Appearance, Color, Odor and Taste

White crystalline powder, practically odorless,

with a slig

3. PHYSICAL PR

3.1 Spectra

3.1.1 Ultraviolet

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1.

Solvent	λm (ε)
Water	2
Methanol	2
Borate buffer	2

3.1.2 Infrared Sp

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given in T.
structure.

IR spec

Wavenumb (cm^{-1})
3380
3240, 3(
2940, 2(
1630

with a slightly bitter taste (6).

3. PHYSICAL PROPERTIES

3.1 Spectra

3.1.1 Ultraviolet Spectrum

The ultraviolet spectrum of Iopamidol (Bracco Working Standard) was determined in water, methanol and borate buffer pH = 9, with a Cary mod.219 spectrophotometer.
The UV spectrum of Iopamidol in water is shown in Fig.1 and some spectral data are presented in Table 1.

Table 1
UV spectral data

Solvent	λmax (nm)	log ε max	$E^{1\%}_{1cm}$ (λmax)		
			mean	s.d.	n
Water	242	4.46	371	0.46	10
Methanol	241	4.47	382	0.61	10
Borate buffer	242	-	380	-	-

3.1.2 Infrared Spectrum

The Infrared spectrum of anhydrous Iopamidol (Working Standard sample) is shown in Fig.2.
The spectrum was obtained on a 0.3% dispersion in KBr with a Perkin Elmer mod.882 Spectrophotometer.
Spectral assignments for principal absorption bands given in Table 2 are consistent with the proposed structure.

Table 2
IR spectral data for anhydrous Iopamidol

Wavenumber (cm^{-1})	Assignment (s)
3380	νOH
3240, 3060	νNH, sec.amide
2940, 2880	νCH, aliphatic
1630	νC=O, amide 1st band

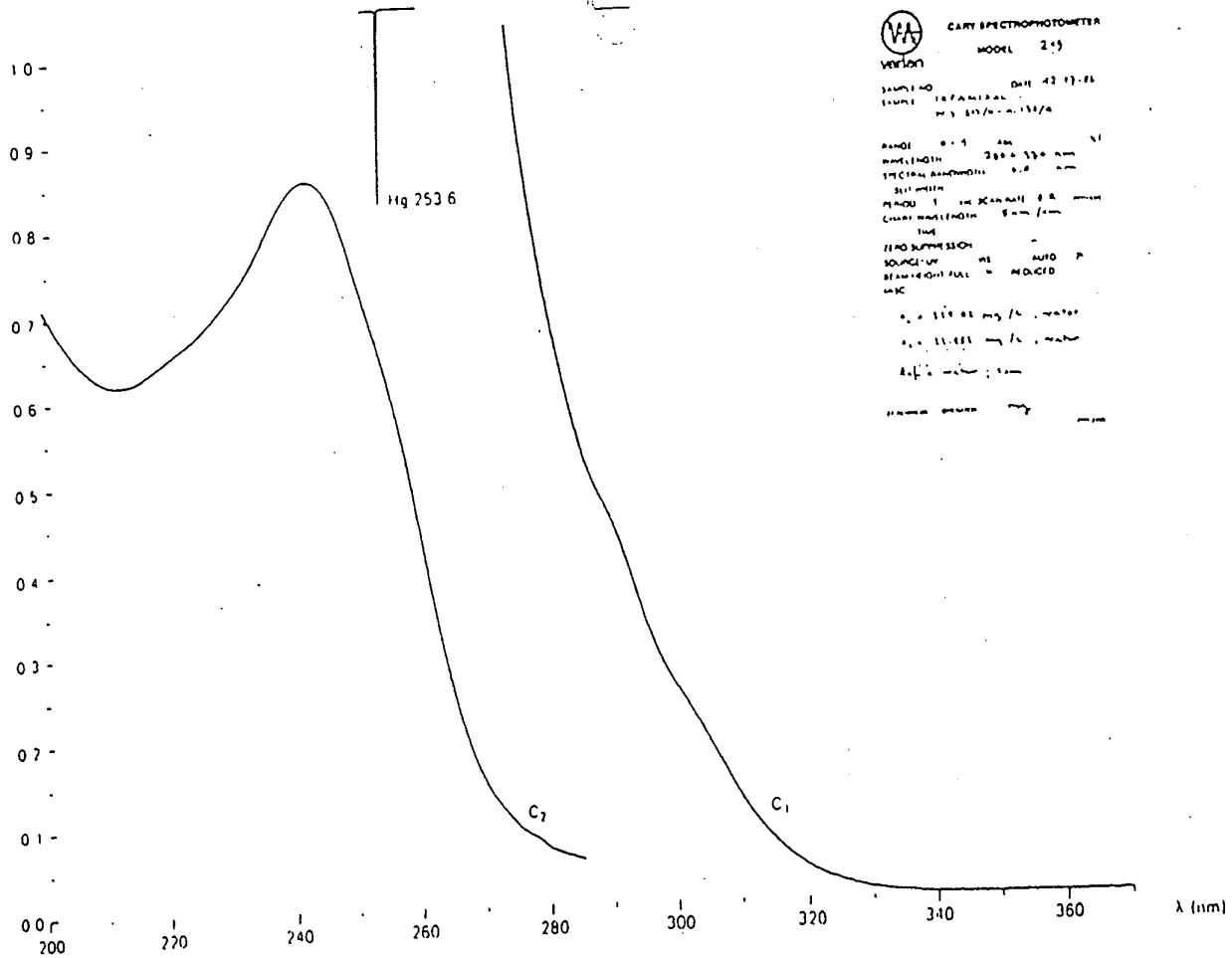
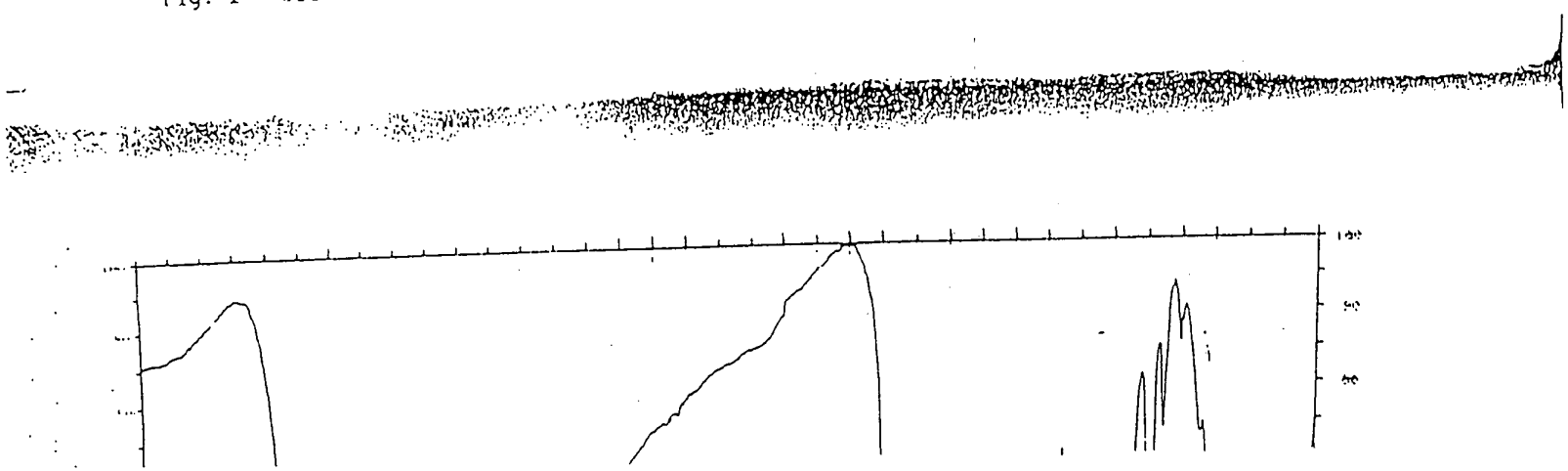


Fig. 1 - Ultraviolet spectrum of Iopamidol in water



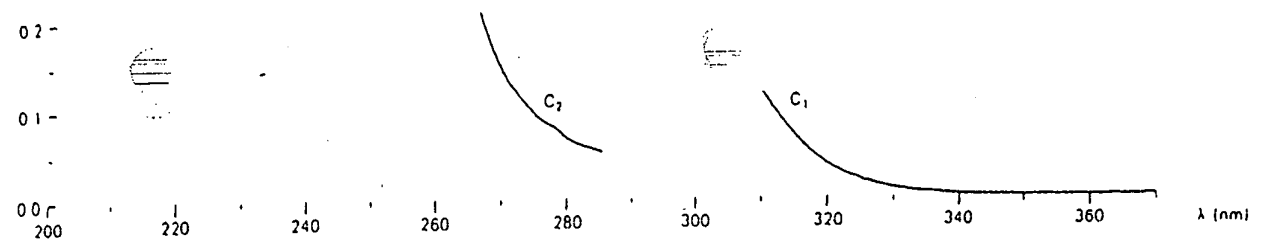


Fig. 1 - Ultraviolet spectrum of Iopamidol in water

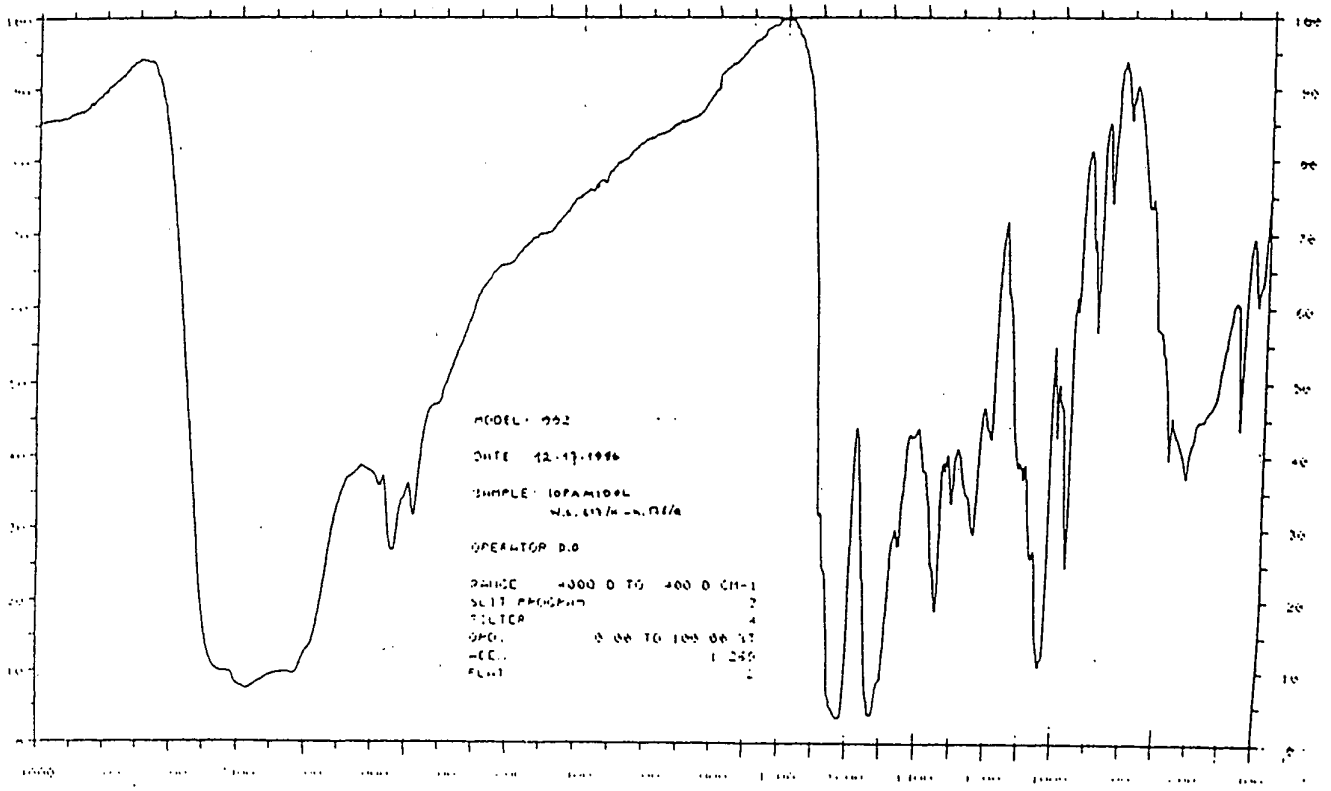


Fig. 2 - Infrared spectrum of Iopamidol in KBr

Wavenumber (cm^{-1})	Assignment (s)
1530	δNH+νCN, amide 2nd band
1350	νCN+δNH, amide 3rd band
1245	δOH
1100	νC-O, primary alcohol
1045	νC-O, secondary alcohol
970	
890	
680	
625	
470	

The two hydrated forms of Iopamidol (see 3.2.1) gave IR spectra with absorption bands that, although consistent with the structure of the product, showed significant differences between each other and the anhydrous form mainly in the region between 1700 and 800 cm^{-1}.

3.1.3 Nuclear Magnetic Resonance Spectra

Numerous isomers are predictable to be present at the equilibrium, due to the possible hindered rotations around the aryl-CO, aryl-N, and N-CO bonds. The detectability of ambient temperature isomers has been proved by Bradamante et al. (7): it has been shown by ^{1}H and ^{13}C NMR analyses that different free energies of activation pertain to the various hindered rotations, allowing discrimination of the effects.
Variable temperature experiments in DMSO-d_6 and D_2O solvents (.04 M) indicate that :

a) isomers derived from the hindrance of rotation around the aryl-CO bond (syn and anti) characterized by whether or not the C=O double bonds point towards the same direction relative to the plane of the benzene ring, are present in the ratio of 1:1;
b) isomers derived from CO-N hindered rotation in the isophthalic carboxamido moiety (E and Z) are present in the ratio of 1:1;
c) rotation around the aryl-N bond is fast on the NMR time scale;
d) a conformational preference for the endo isomer is detectable in the case of isomers derived from the CO-N hindered rotation in the anilido moiety (endo and exo).

3.1.3.1 ^{1}H-NMR

^{1}H NMR
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^{1}H-NM

Chemical shift δ_H (ppm,TMS)
9.69
8.18 }
7.62 }
5.65
4.68
4.52
4.18
3.87
3.7-3.4
1.39

3.1.3.1 ^{1}H-NMR

^{1}H NMR spectra of Iopamidol (Bracco Working Standard) were recorded in DMSO-d_6 and D_2O solutions with a Bruker AC-200 Spectrometer operating at 200 MHz (8).
Chemical shifts and assignments are reported in Tables 3 and 4, while Figures 3 and 4 show the normal spectrum in DMSO and the 2D spectrum (COSY-90) respectively, further confirming the multiplicities observed and their assignments.

Table 3

^{1}H-NMR data (200 MHz) in DMSO-d6

Chemical shift δH (ppm,TMS)	Multiplicity	nr.protons	Assignments (s)
9.69	s	1 exch.	6-NHCO
8.18 7.62	b,m b,m	2 exch.	2 6-CONH
5.65	m	1 exch.	CH_3-CH(OH)
4.68 4.52	b,t b,t	1 exch. 3 exch.	4 -CH_2OH
4.18	m	1	CH_3-CH(OH)
3.87	b,m	2	2 -CH-N
3.7-3.4	m	8	4-CH_2OH
1.39	d	3	CH_3-CH(OH)

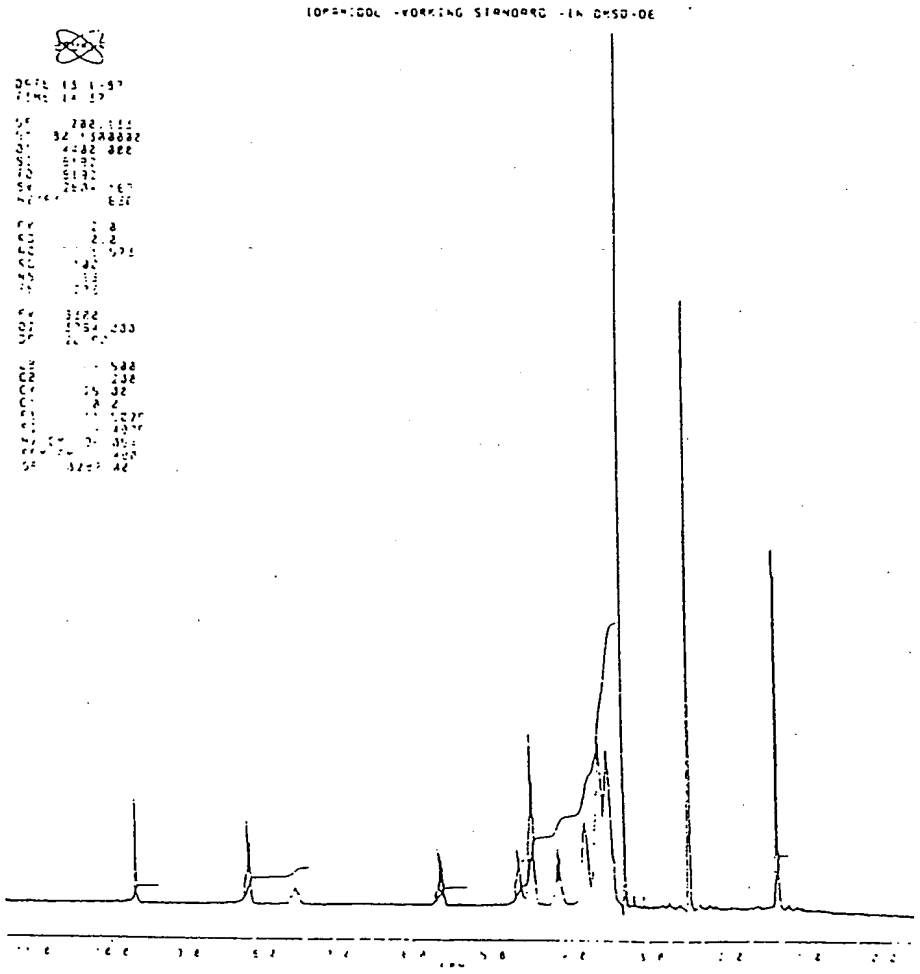

Fig. 3 - ^{1}H-NMR spectrum of Iopamidol in DMSO

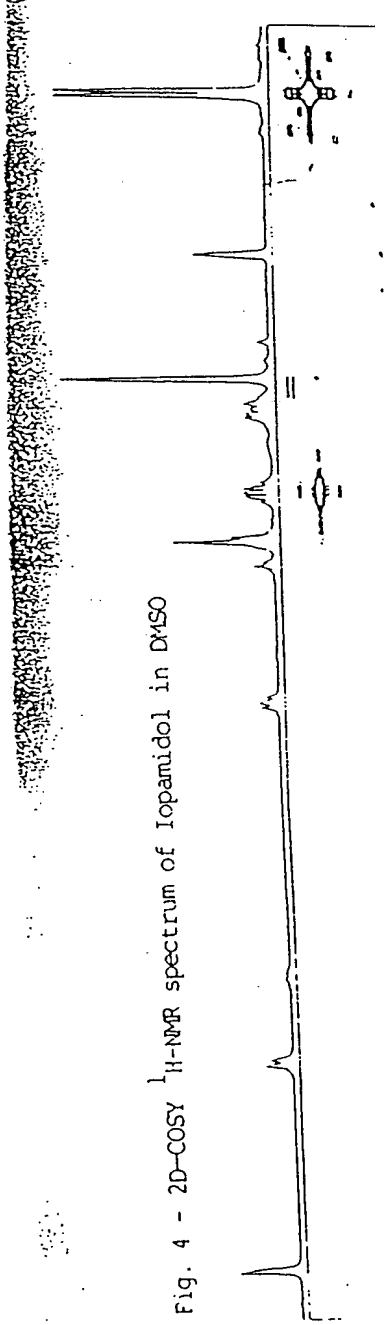

Fig. 4 - 2D-COSY ^{1}H-NMR spectrum of Iopamidol in DMSO

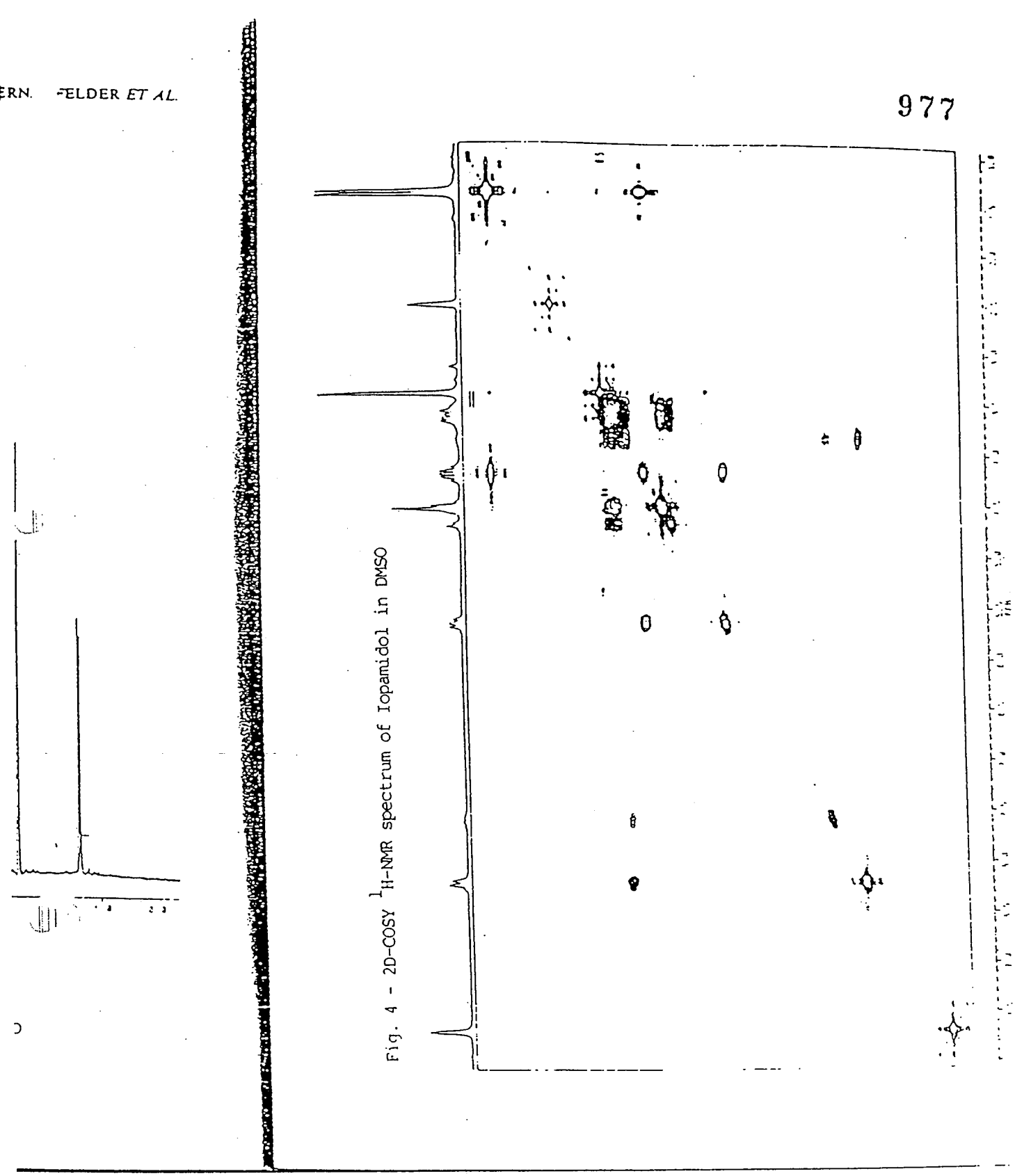

Fig. 4 - 2D-COSY ^{1}H-NMR spectrum of Iopamidol in DMSO

Table 4

^{1}H-NMR data (200 MHz) in D_2O

Chemical shift δ_H (ppm) (*)	Multiplicity	nr.protons	Assignment(s)
4.49	q	1	CH_3-CH(OD)
4.16	qui	2	2-CH-N
3.82	d	8	4-CH_2OD
1.55	d	3	CH_3-CH(OD)

(*) corrected for TMS

3.1.3.2 ^{13}C-NMR

^{13}C-NMR spectrum of Iopamidol (Bracco Working Standard) shown in Figure 5 was recorded in DMSO-d_6 solution with a Bruker AC-200 spectrometer at 50 MHz (8). Chemical shifts and assignments are reported in Table 5.

3.1.4 Mass Spectrum

The remarkably low volatility of Iopamidol, due to its high molecular weight and to the presence of several polar functional groups, complicates its mass spectral characterization.
Partial information has been gained by chemical derivatization procedures combined with different ionization methods and conclusive mass spectral characterization of Iopamidol was obtained when the fast atom bombardment (FAB) technique was employed (9).
The FAB mass spectrum of Iopamidol in glycerol is shown in Fig. 6.
As can be observed the base peak corresponds to the protonated molecular ion (m/z 778).
The fragmentation pattern of this $(M+H)^+$ species is shown in Scheme 1.

Assignment(s)
CH_3-CH(OD)
2-CH-N
4-CH_2OD
CH_3-CH(OD)

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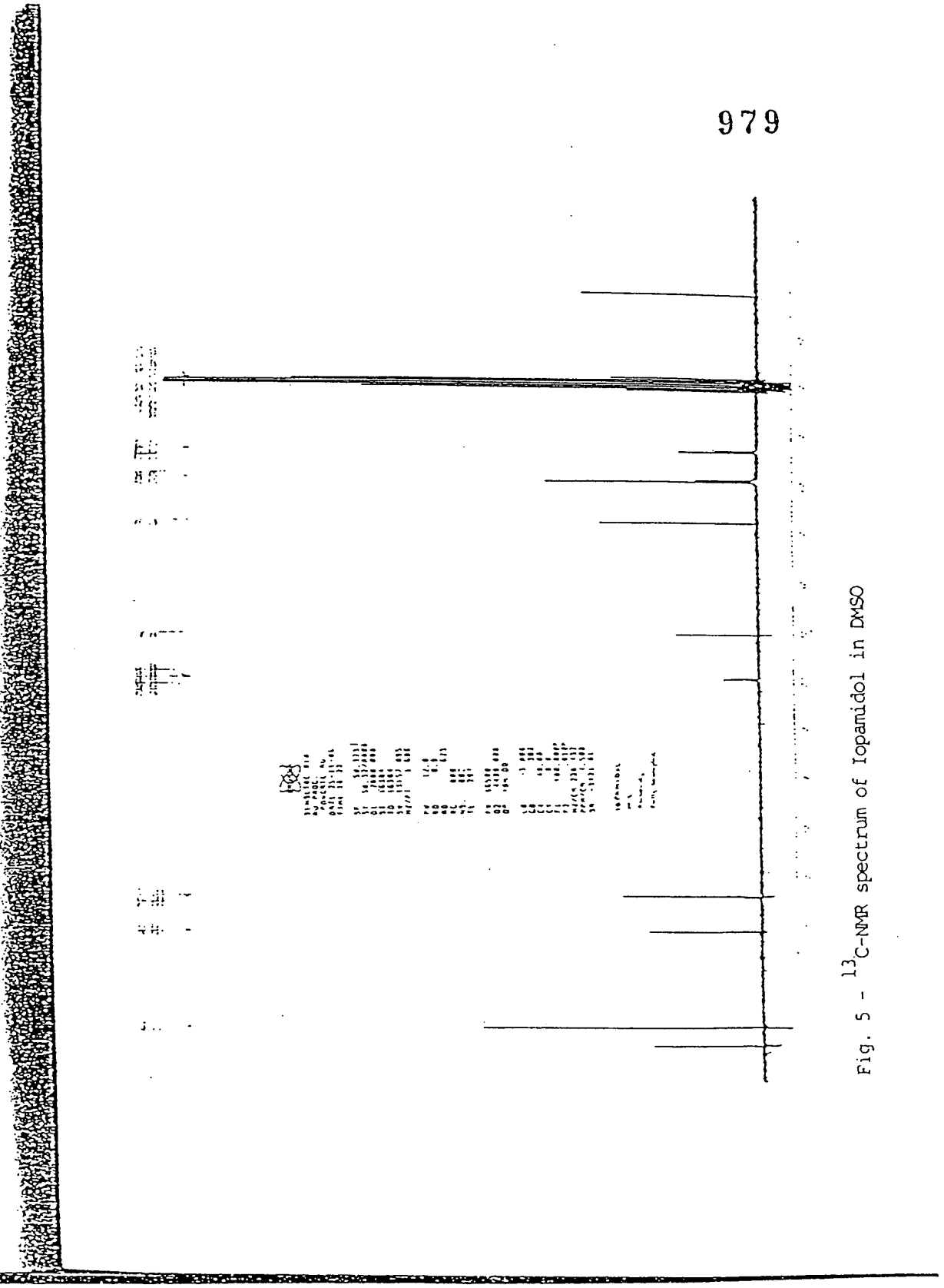

Fig. 5 - ^{13}C-NMR spectrum of Iopamidol in DMSO

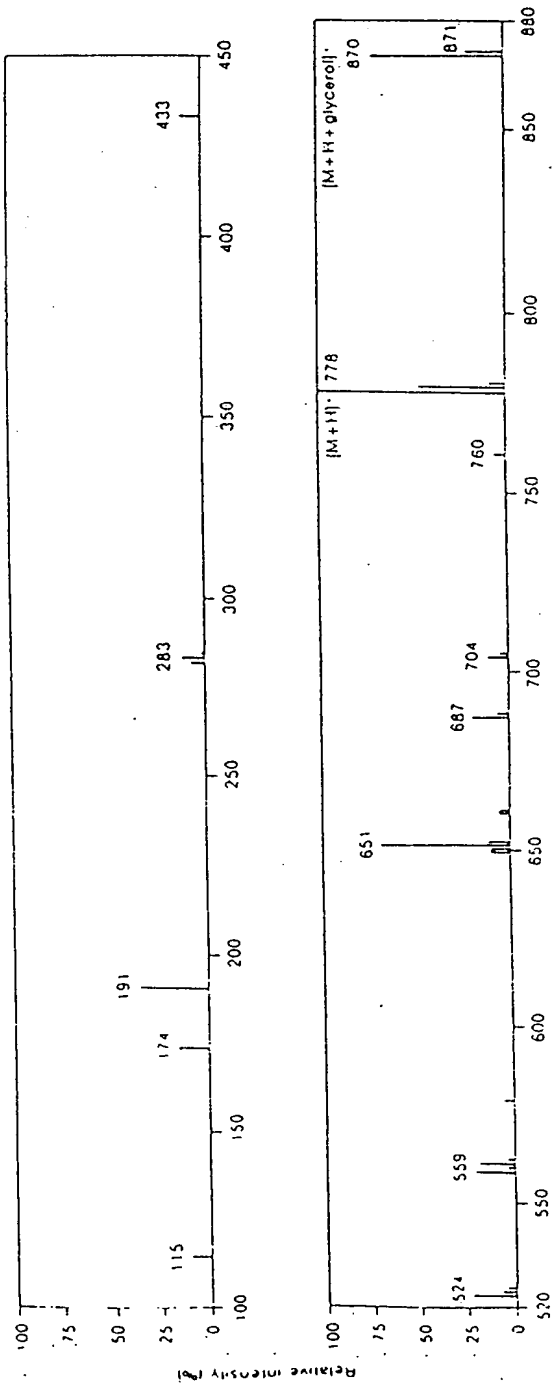

Fig. 6 - FAB MASS spectrum of Iopamidol

IOPAMIDOL

^{13}C-NM

Chemical shi. δ_C (ppm, TM:
172.67
169.06
168.89
149.82
149.64
143.10
142.79
142.72
99.20
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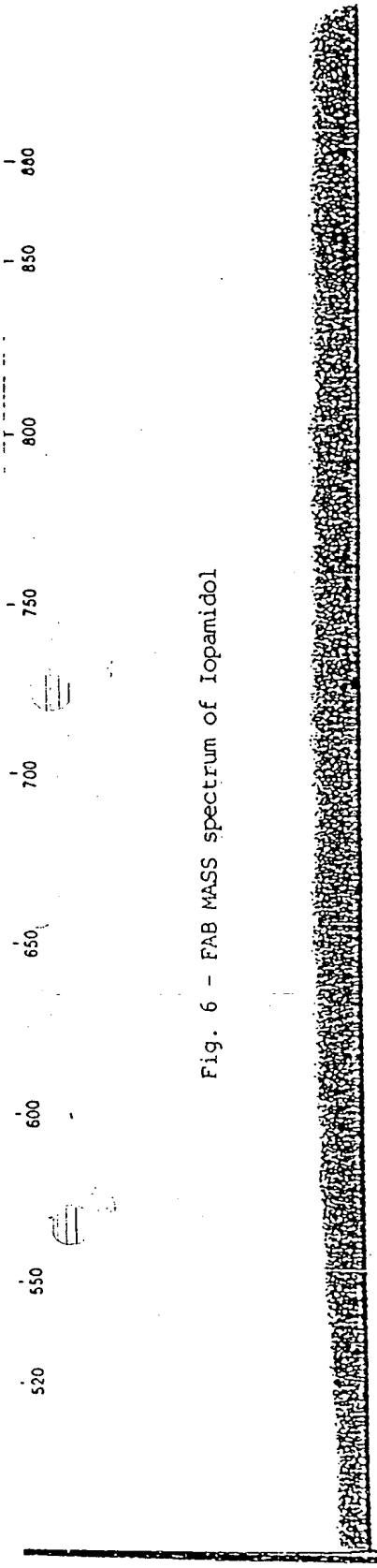


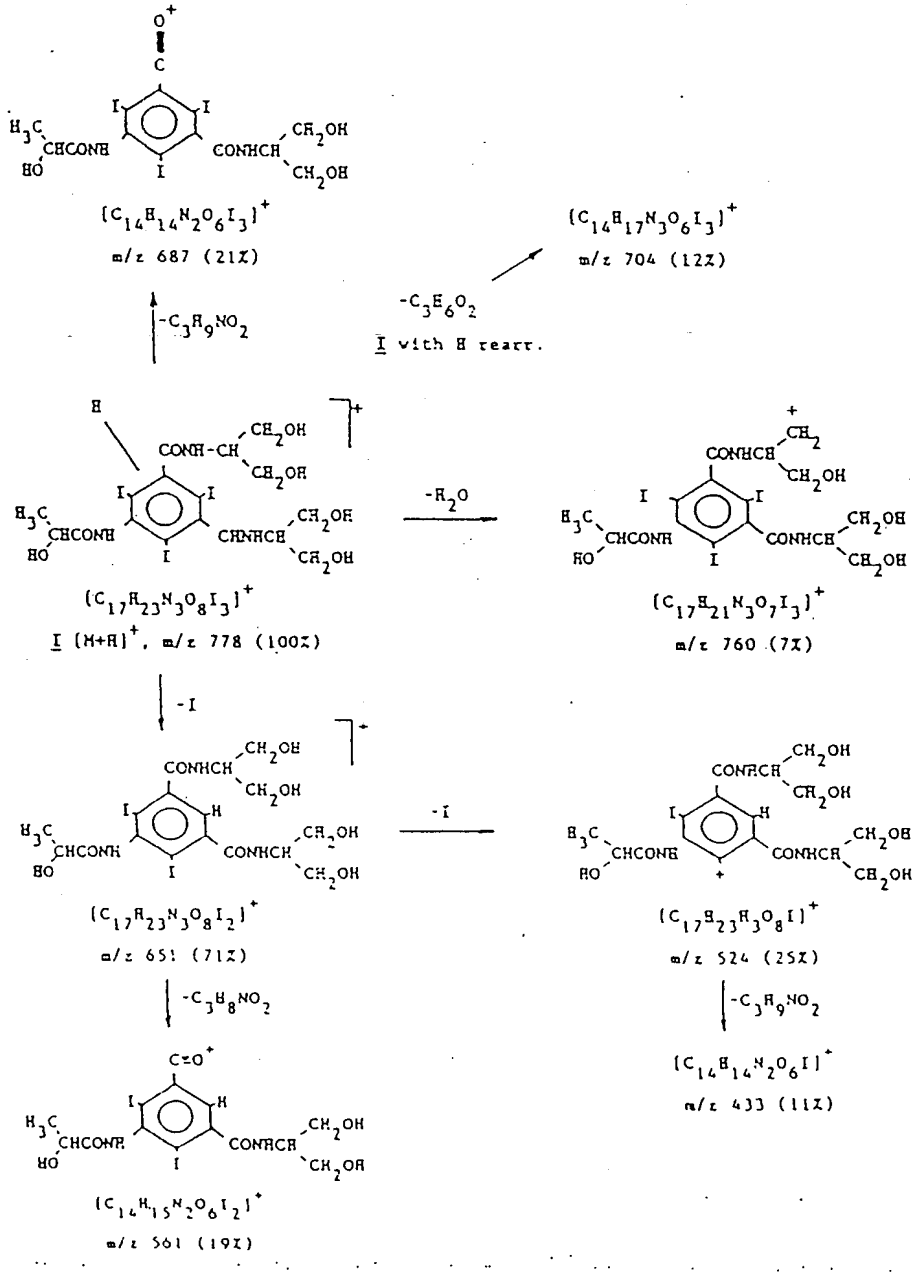
Fig. 6 - FAB MASS spectrum of Iopamidol

Table 5

^{13}C-NMR data (50 MHz) in DMSO-d6

Chemical shift δ_C (ppm, TMS)	Assignment (s)
172.67	ǿNHCO
169.06 168.89	ǿCONH
149.82 149.64	Aromatics C-3, C-5
143.10 142.79 142.72	Aromatic C-1(N)
99.20 99.06 98.97 98.91 98.77	Aromatics C-2, C-6
90.07	Aromatic C-4
67.59	CH, lactoyl
59.28 58.86	CH_2, serinol
53.19 53.10 52.78	CH, serinol
22.11	CH_3

SCHEME 1
FRAGMENTATION PATTERN OF IOPAMIDOL



3.2 Solid state

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Parameter
a (A)
b (A)
c (A)
α (degr.)
β (degr.)
γ (degr.)
V (A^3)
Dx(g/cm^3)
Z
Space group

Structural
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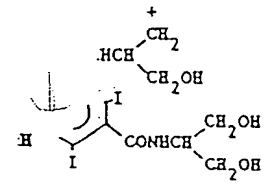
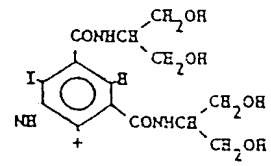
and penta-
and SYN
isophthali

$N_3O_6I_3]^+$
4 (12%)



$[C_{17}H_{21}N_3O_7I_3]^+$
m/z 760 (7%)



$[C_{17}H_{23}N_3O_8I]^+$
m/z 524 (25%)

$C_3H_9NO_2$

$_{14}N_2O_6I]^+$
m/z 433 (11%)

3.2 Solid state properties

3.2.1 Crystal morphology

Iopamidol exists in three different crystal forms, corresponding to the anhydrous, the monohydrate and the pentahydrate, each characterized by a distinct IR Spectrum (see 3.1.2), X-ray powder diffraction pattern (see 3.2.2) and by distinct both enthalpimetric (see 3.2.4) and gravimetric (see 3.2.5) thermograms.
Crystals of the three forms, which were induced to grow with great difficulty from aqueous solutions, were subjected to structural X-ray analysis using a Philips PW 1100 diffractometer (Mo K α = 0.71069 Å; scan speed = 0.06°/s) for the anhydrous form and a CAD-4 diffractometer for the mono and the penta-hydrate forms.
Crystal cell parameters of each of the three forms are reported in Table 6.

Table 6
Crystal data of Iopamidol

Parameter	Anhydrous (10)	Monohydrate (11)	Pentahydrate (12)
a (A)	12.478	15.591	14.147
b (A)	11.233	13.611	12.484
c (A)	9.241	12.608	15.963
α (degr.)	104.49	81.41	90.00
β (degr.)	92.63	62.25	90.69
γ (degr.)	108.63	87.10	90.00
V (A^3)	1117	2426	2819
Dx(g/cm^3)	2.19	2.18	2.04
Z	2	4	4
Space group	P_1	P_1	P_{21}

Structural analysis of the anhydrous and of the pentahydrate forms further confirmed what was observed by NMR spectroscopy (see 3.1.3) as regards the ENDO conformation of the CO-N bond (anhydrous and penta- hydrate) and the ANTI E,E (pentahydrate) and SYN E,E (anhydrous) conformations of the isophthalic bonds Ø-CONHR.

3.2.2 X-Ray Powder Diffraction

X-Ray powder diffraction patterns of the three forms of Iopamidol were obtained with a Philips PW 1710 diffractometer in the 2θ range between 3 and 50° using a Cu-Ni radiation (40 KV; 40 mA) and a 1°/min scanning rate (13). Data obtained on samples crystallized from water are reported in Tables 7, 8 and 9 and clearly demonstrate that the three forms yield three distinct diffractograms.

Table 7
X-ray Powder Diffraction Pattern of anhydrous Iopamidol

D (Å)	I% rel.	D (Å)	I% rel.
11.73	100	2.99	22
10.24	40	2.94	22
9.49	34	2.90	39
8.88	24	2.88	27
7.89	49	2.85	41
7.61	27	2.79	20
6.66	33	2.74	14
6.04	35	2.73	14
5.86	19	2.69	46
5.10	58	2.62	16
5.05	74	2.58	32
4.73	72	2.54	13
4.56	97	2.43	12
4.46	17	2.41	12
4.27	42	2.37	18
4.23	31	2.34	24
4.18	33	2.27	16
4.15	22	2.25	12
3.97	32	2.23	19
3.94	37	2.21	21
3.90	66	2.19	13
3.80	31	2.17	17
3.77	21	2.11	15
3.71	37	2.09	16
3.61	33	2.07	14
3.58	44	1.99	13
3.52	37	1.96	14
3.42	17	1.88	16
3.40	14	1.85	16
3.29	42		
3.15	16		
3.08	51		

X-Ray Powder Di

D (Å)
13.48
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3.41
3.32
3.27
3.17

Table 8

X-Ray Powder Diffraction Pattern of monohydrate Iopamidol

D (Å)	I%rel.	D (Å)	I%rel.
13.48	14	3.11	14
11.38	50	3.07	8
9.68	100	3.04	10
8.19	14	3.01	7
7.15	12	2.97	7
6.88	9	2.94	9
6.32	41	2.88	11
6.16	12	2.86	15
6.05	11	2.84	9
5.72	46	2.80	10
5.53	13	2.76	20
5.49	19	2.73	20
5.28	12	2.61	19
5.23	12	2.54	6
5.03	11	2.50	10
4.86	33	2.46	7
4.69	8	2.44	6
4.60	8	2.37	6
4.56	12	2.34	5
4.49	12	2.32	5
4.26	14	2.27	7
4.20	12	2.26	6
4.10	10	2.20	6
3.99	20	2.13	8
3.87	6	2.11	9
3.80	17	2.09	8
3.70	11	2.05	15
3.67	9	2.00	20
3.57	17	1.95	5
3.52	10	1.86	7
3.44	15	1.70	5
3.41	13	1.65	6
3.32	11	1.62	9
3.27	16	1.62	6
3.17	8		

Table 9

X-Ray Powder Diffraction Pattern of pentahydrate Iopamidol

D (Å)	I%rel.	D (Å)	I%rel.
14.12	74	3.16	15
10.62	29	3.08	72
9.79	51	3.03	13
9.33	51	2.90	25
8.35	29	2.87	36
7.78	36	2.85	57
7.33	40	2.82	100
7.06	30	2.79	25
6.71	83	2.77	33
6.59	60	2.71	36
6.23	36	2.69	20
6.14	34	2.65	25
5.82	21	2.60	30
5.70	20	2.57	24
5.53	45	2.54	18
5.14	38	2.51	15
4.98	57	2.45	27
4.89	24	2.42	22
4.79	44	2.40	15
4.62	35	2.31	17
4.58	20	2.27	12
4.46	30	2.25	19
4.38	67	2.22	25
4.29	22	2.18	15
4.18	16	2.14	26
4.03	44	2.09	23
3.98	35	2.06	20
3.89	20	1.99	25
3.83	29	1.95	21
3.75	33	1.93	21
3.67	86	1.92	18
3.59	18	1.90	24
3.57	17	1.88	18
3.44	13	1.84	12
3.38	59	1.81	16
3.35	28	1.74	13
3.27	39	1.71	13
3.22	34	1.69	16
3.19	30	1.56	14

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3.2.3 Melting Ra

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3.2.3. Melting Range and Eutectic Temperature

Melting point determination of anhydrous Iopamidol, alone or in mixture with suitable substances, was made with a Kofler microscope operating as described by Roth (14). The product shows no change up to a temperature of 290°; then the crystal becomes black and a slow decomposition begins.
In mixtures with benzil, acetanilide, phenacetine and benzanilide, the compound does not change the m.p. of the mixed substances while it lowers the m.p. of salophen (186°; $\Delta T = 2.6°$) and cyanoguanidine (180°; $\Delta T = -28.7°$).

3.2.4 Differential thermal analysis

Differential thermal analysis was carried out using a Mettler TA 3000 calorimeter in the range 30-300°C with a heating rate of 10°/min and a purge (air) of about 25 ml/min (15). Figure 7 shows the thermograms of the anhydrous, monohydrate and pentahydrate forms of Iopamidol which exhibit three clearly distinct patterns characterized as follows :

- anhydrous form : monotonous curve with a wide (140-220°) endothermic transition of small intensity

- monohydrate form : three endothermic transitions at 115° ($\Delta H \simeq 55$ J/g), 250° and 265° the latter two not resolved, with ΔH of about 40 and 67 J/g. The first transition is associated with the loss of one molecule of water.

- pentahydrate form : four endothermic transitions at 87° ($\Delta H \simeq 215$ J/g), 117° ($\Delta H \simeq 55$ J/g), 183° ($\Delta H \simeq 10$ J/g)and 255° ($\Delta H \simeq 30$ J/g). The first two transitions are ascribable to loss of 4 and 1 moles of water, respectively.

Above 300° all the forms undergo decomposition with release of iodine.

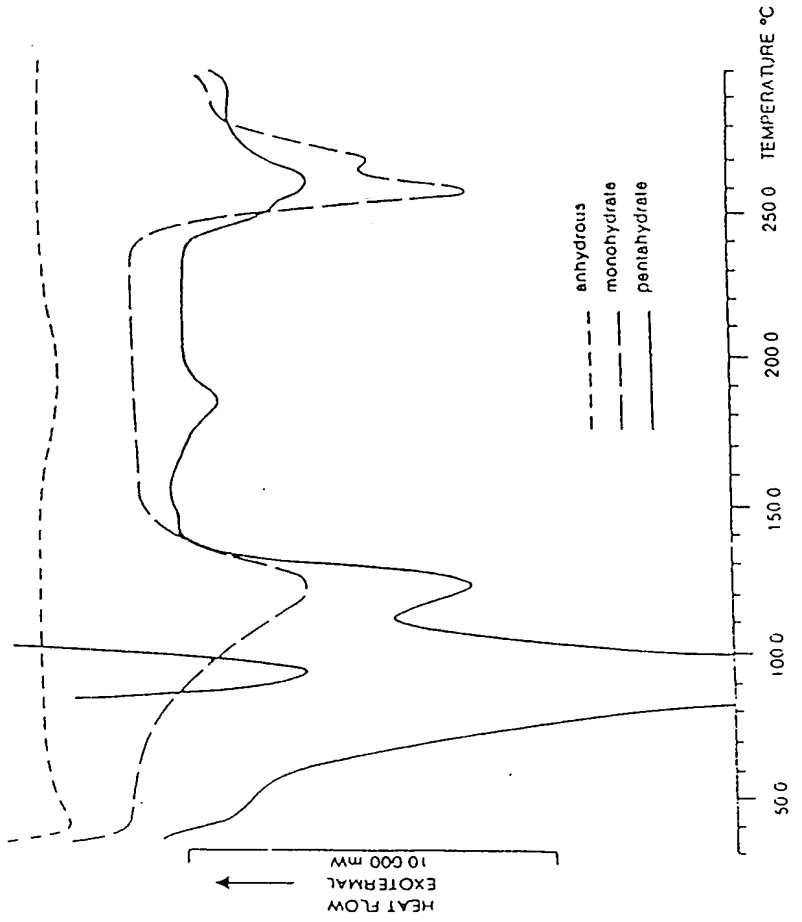

Fig. 7 - DTA of various forms of Iopamidol

IOPAMIDOL

3.2.5 Thermogravi

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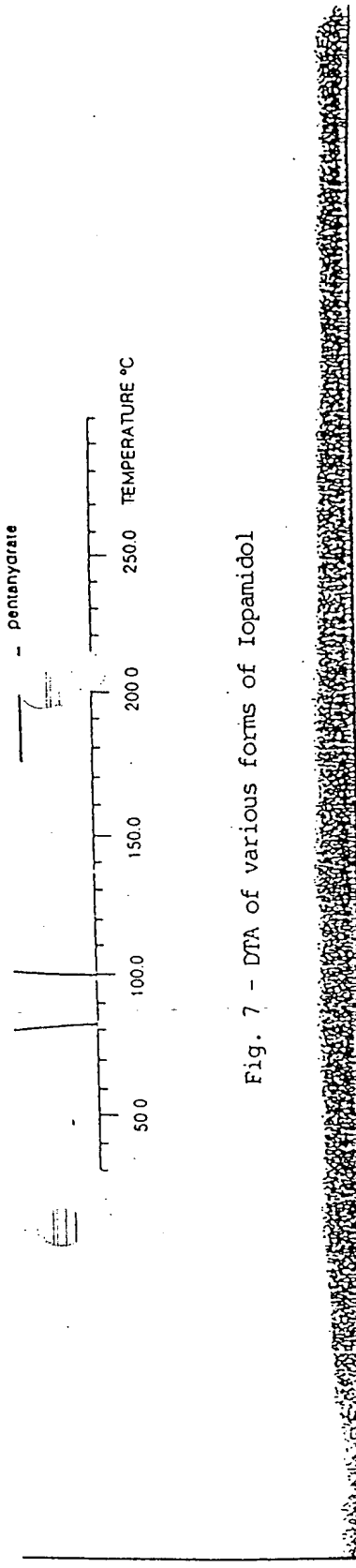


Fig. 7 - DTA of various forms of Iopamidol

3.2.5 Thermogravimetric analysis

Thermogravimetric analysis of the anhydrous, the monohydrate and the pentahydrate forms of Iopamidol were carried out using a Mettler apparatus consisting of a thermobalance TG50, a TA processor TC10A and a printer, in the temperature range 30-200° and with a heating rate of 10°/min (15). The results showed that the monohydrate form loses 2.0% (0.9 moles of water) of its weight around 108°, the pentahydrate 8.1% (4 moles of water) at 80° and another 2.3% (1 mole of water) at about 104°, while the anhydrous form remains unchanged. The behaviour observed is consistent with what deduced from differential thermal analysis.

3.3 Solution properties

3.3.1 Optical rotation

Optical rotation of an aqueous solution of Iopamidol was studied by Felder (16), who reported that optical rotation of a 10% aqueous solution is quite low: $[\alpha]20°,589nm = -3.20° \pm 0.01°$ and scarcely influenced by temperature : $[\alpha]T,589\ nm = -2.762° \pm 0.022\ T \pm 0.010°$ ($20° < T < 40°$). In the region 589 ÷ 365 nm it resulted that the optical rotatory dispersion curve could be expressed by the single equation of Drude : $[\alpha]20,\lambda = -0.6871°/\lambda^2 - 0.056 \pm 0.024$ (λ = μm). Moreover, the Author described a method for the determination of optical purity of the product by enzymatic reaction with L- and D-lactic dehydrogenase following reductive dehalogenation of Iopamidol and subsequent alkaline hydrolysis, and also the possibility of increasing considerably the specific rotatory power of Iopamidol by complexation with Cu (II) ions in presence of alkali. A value of $[\alpha]20°,436nm = 142.2° \pm 0.11°$ (c=2.5%; water) was found for the complex ML2 using a product with a purity corresponding to 98.9 ± 0.5% of L form and 1.62 ± 0.19% of D form.

3.3.2 Solubility in water

The question of the water-solubility of Iopamidol cannot be univocally answered.
In fact, in the same range of temperatures, different solubility curves exist according to the re-

spective nature of the crystalline phases at the equilibrium (Fig.8). In the absence of any crystalline phase at the start, even solutions of extremely high concentrations (>>400 mgI/ml), while being proportionally more viscous up to the limit of vitreous consistency, can last indefinitely without ever forming a new phase.
Three crystalline phases, different as to their lattice spacings, molecular conformations, and degrees of hydration, have been isolated and characterized with various techniques (see 3.1.2, 3.2.1, 3.2.2, 3.2.4, 3.2.5) and their respective solubility curves are depicted in Fig.8.

3.3.2 Ionization constant

The acid dissociation constant of Iopamidol was determined by potentiometric titration in water (17). The pKa value is 10.70 at 25°. Accordingly, the pH of an unbuffered molar aqueous solution is 5.3 and the degree of ionization at pH = 7.0 is only 0.02%.

3.3.4 Partition coefficient

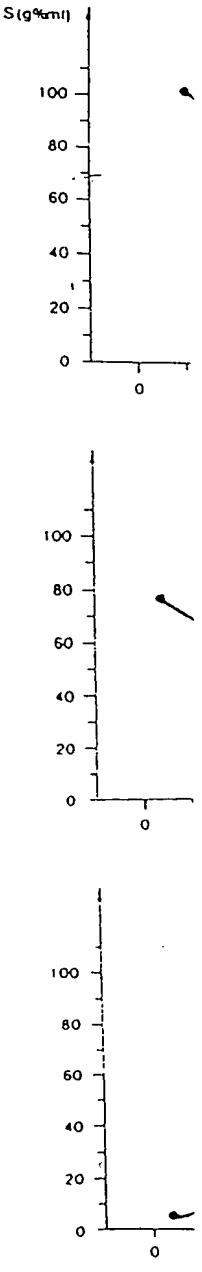
Partition coefficients were determined at 20° in n-octanol/0.01M phosphate buffer pH 7.4 and in n-butanol/0.01M phosphate buffer pH 7.4 according to Leo (18).
The values found are given in Table 10.

Table 10
Partition coefficients at pH 7.4 and 20°

Organic phase	P ± sd
n-Octanol	0.0025 ± 0.0001
n-Butanol	0.094 ± 0.005

3.3.5 Density 20/20°

Densities of aqueous solutions of Iopamidol were measured at 20° using a 1 ml Pregl pipette (14).
In the concentration range c = 10-80 g% ml the



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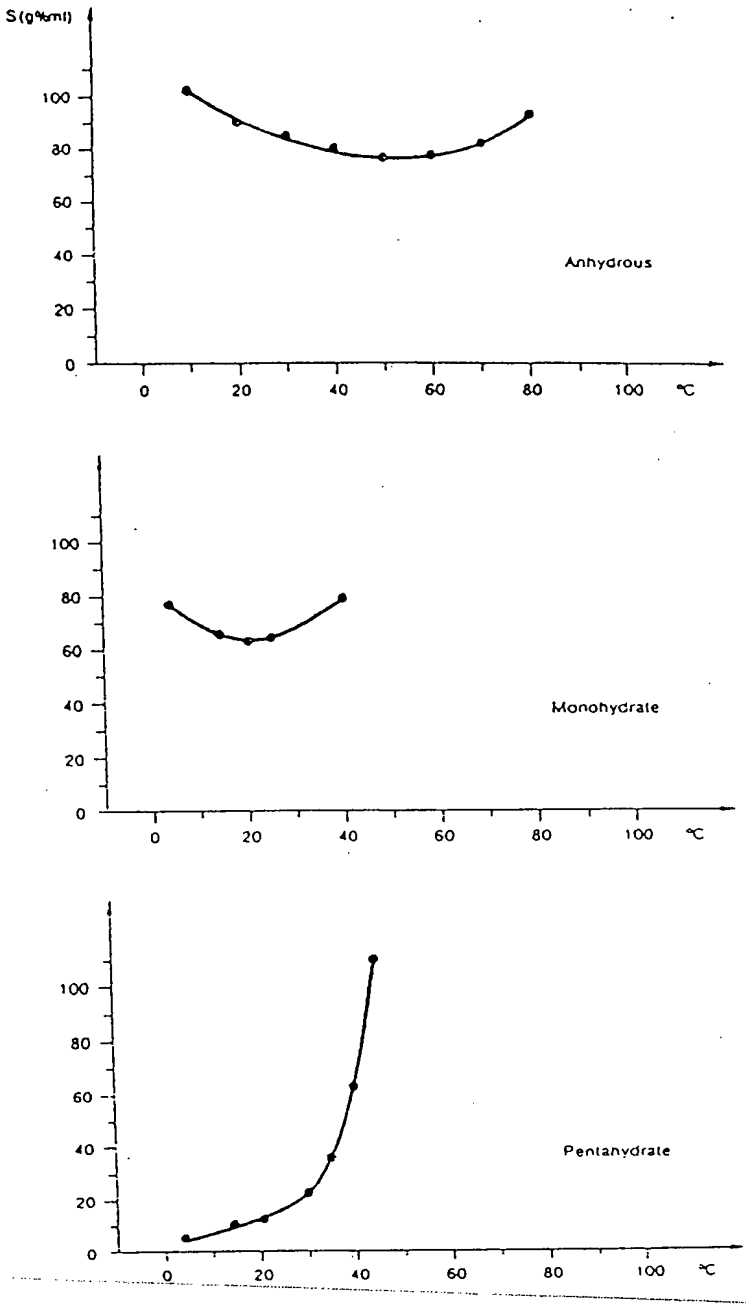

following relationship was found :
d 20/20 = 0.99669 + 0.0055033 . c ± 0.00035 ;
r=0.999997.

3.3.6 Refractive index

The refractive index of a series of aqueous solutions of Iopamidol was determined at 20° with an Abbe-ATAGO refractometer.
The following relationship was found in the c = 10 ; 80g% ml concentration range : n 20°, D = 1.33240 + 0.0016386 . c ± 0.00055; r=0.999984.

3.3.7 Viscosity

Viscosity measurements of a series of aqueous solutions of Iopamidol were carried out at 20° and at 37° using a Haake Rotovisco RV-100 Viscosimeter. Before testing, aqueous solutions were filtered through a 0.45 µm Millipore membrane and sterilized at 120° for 30 minutes.
Table 11 lists the mean values found (19).

Table 11

Iopamidol viscosity data

Concentration (mgI/ml)	η Viscosity. ($mP.s^{-1}$)	
	20°	37°
100	1.6	1.1
150	2.3	1.5
200	3.3	2.1
250	5.0	3.1
300	8.3	4.4
370	18.4	9.0

The relationship between viscosity and temperature can be expressed by the following exponential equations :

$\eta^{20} = 0.593 \cdot e^{0.009.c}$; r = 0.989

$\eta^{37} = 0.475 \cdot e^{0.008.c}$; r = 0.991

3.3.8 Osmotic pr

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Concentra	
mol/l	mol/kg
0.263	0.291
0.394	0.459
0.525	0.646
0.657	0.857
0.788	1.092
0.972	1.479

(*) does not f

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Concentrat	
mol/L	mol/Kg
0.263	0.291
0.394	0.459
0.525	0.646
0.657	0.857
0.788	1.092
0.972	1.479

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3.3.8 Osmotic properties

Osmolality, osmotic pressures and osmotic coefficients of a series of aqueous solutions of Iopamidol, previously sterilized at 120° for 30 min, were determined both by cryoscopy (Advanced Mod 3 W II, Advanced Instruments) and by vapor pressure measurements (Knauer, vapor pressure osmometer); in the latter case measurements were carried out at 37° after calibrating the instrument with aqueous mannitol solutions (20). Values are reported in Table 12 and 13.

Table 12
Iopamidol Osmometric data by cryoscopy

Concentrations				Values found (23)		
mol/l	mol/kg	g%ml	mgI/ml	φ_{vm} (osmol/kg)	π (atm)	γ
0.263	0.291	20.4	100	0.236	6.01	0.81
0.394	0.459	30.6	150	0.346	8.81	0.75
0.525	0.646	40.8	200	0.465	11.8	0.72
0.657	0.857	51.1	250	0.594	15.4	0.69
0.788	1.092	61.2	300	0.740	18.8	0.68
0.972	1.479	75.5	370	(*)	-	-

(*) does not freeze

Table 13
Vapor pressure osmometric data of Iopamidol at 37°

Concentrations				Values found		
mol/L	mol/Kg	g%ml	mgI/ml	φ_{vm} (*) (osmol/Kg)	π (**) (atm)	γ (***)
0.263	0.291	20.4	100	0.224	5.70	0.77
0.394	0.459	30.6	150	0.318	8.09	0.69
0.525	0.646	40.8	200	0.416	10.6	0.64
0.657	0.857	51.1	250	0.513	13.1	0.60
0.788	1.092	61.2	300	0.620	15.8	0.57
0.972	1.479	75.5	370	0.799	20.3	0.54

(*) Osmolality = $\ln a / v_o d_o$ (21,22)
(**) Osmotic pressure = $\varphi_{vm} RT$
(***) Osmotic coefficient

The explanation of the fact that the cryoscopic method yields higher values, lies in the non-ideal behaviour of Iopamidol solutions. Studies carried out on 0.1 ÷ 1.5 mol/kg Iopamidol solutions using the classic cryoscopic method for freezing point determination (24), confirmed this tendency and also showed that the composition of the solid phase which separates upon freezing, e.g. from a solution at 370 mgI/ml, is virtually unchanged with respect to the starting solution. A similar behaviour was previously observed by Børdalen (21) in his study on the osmotic properties of aqueous solutions of conventional contrast media.
For these reasons we consider that, for this class of compounds, data obtained with the vapor pressure method are more reliable.

3.3.9 Surface Tension

The surface tension at 20° of a series of Iopamidol aqueous solutions was determined using an interfacial tensiometer according to Lecomte Du Nouy (Krüss).
Values recorded immediately after preparation of the solutions (time zero) and after an equilibrium period of 16 hours are reported in Table 14.

Table 14

Surface tension at 20°

Iopamidol concentration			Surface tension (dyne/cm)	
g%ml	mgI/ml	mol/L	Start	Equilibrium
10.2	50	0.131	68.7	55.4
20.4	100	0.263	65.3	52.5
40.8	200	0.525	60.7	51.1
61.2_S	300	0.788	59.0	50.8
81.6_S	400	1.051	58.3	50.6

3.9.10 Critical Micelle Concentration (c.m.c.)

Critical micelle concentration value of Iopamidol

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4. SYNTHESIS

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	Equilibrium
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lue of Iopamidol

in water was determined according to the method of "spectral variation" described by Thoma (25) using Eosin Yellowish, Rodamine 6G and Pinacyanol Chloride. The results are listed in Table 15.

Table 15

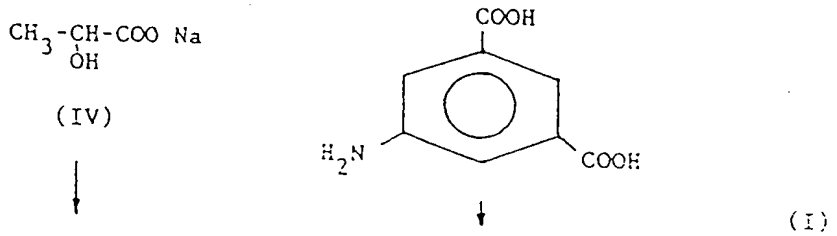
Critical micelle concentration

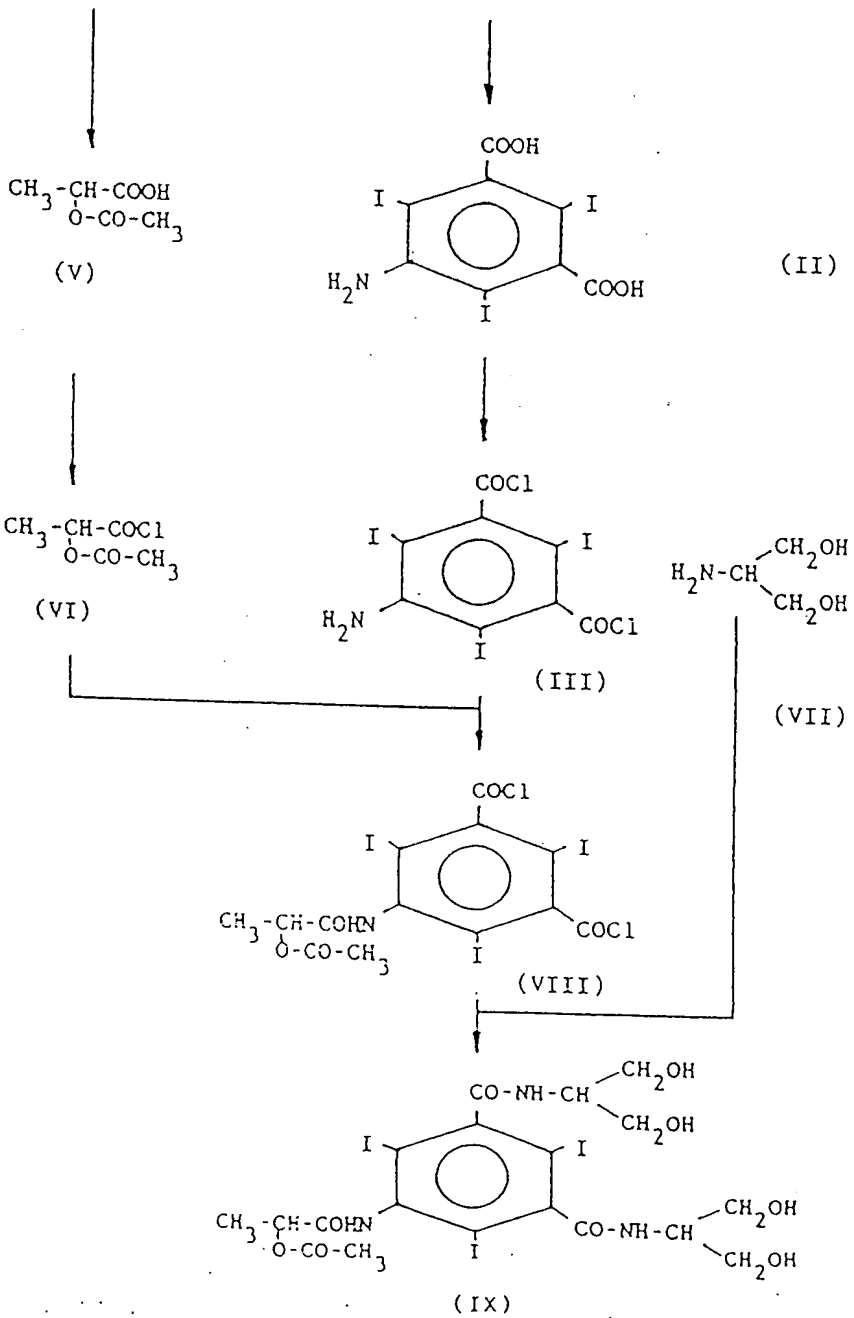
Dyestuff	Analytical (nm)	c.m.c.	
		g% ml	mol/L
Eosine Yellowish	532.0	3.8	$4.9.10^{-2}$
Rodamine 6G	543.5	3.8	$5.0.10^{-2}$
Pinacyanol	619.0	2.8	$3.6.10^{-2}$

4. SYNTHESIS

In Iopamidol synthesis, which was developed by Felder (3) and is outlined in Scheme 2, the starting product aminoisophtalic acid (I) is first iodinated at the 2,4 and 6 positions (II) and then its dichloride (III) is subjected to N-acylation with the chloride of the O-acetyl-L-lactic acid (VI), to condensation with 2-aminopropan-1,3-diol (VII) (Serinol) and finally transformed into the final product (X).
During development of the synthesis on the industrial scale, each single step was further studied as to its kinetics and was optimized in order to reduce to a minimum the possibility of by-products formation and of racemization.

SCHEME 2
Iopamidol Synthesis





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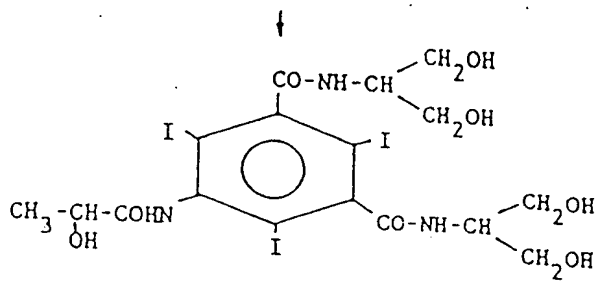

(X)

5. STABILITY AND DEGRADATION

Under normal storage conditions Iopamidol is stable at room temperature.
The main potential degradation routes of the product in its injectable formulation were reported by Felder (26).

6. METHODS OF ANALYSIS

6.1 Elemental analysis

Element	%calc.	%found
C	26.27	26.40
H	2.85	2.94
N	5.41	5.66
I	49.00	48.98
O	16.47	16.22

6.2 Identification tests

The identification of Iopamidol can be carried out according to the following methods :

a) heating on a flame about 50 mg of product in a tube : violet iodine vapors are evolved.

b) the UV spectrum of a solution containing about 20 mg/l of product in pH=9 phosphate buffer, shows an abosorption maximum at 242 nm (E 1%, 1 cm = 380).

c) the IR spectrum of the product in KBr pellet shows characteristic absorption bands at : 3380, 3240, 2940, 2880, 1630, 1530, 1350, 1245, 1045, 970 and 890 cm^{-1}.

6.3 Organically bound iodine

Organically bound iodine is determined potentiometrically in acidic solution by titration with 0.1N $AgNO_3$ after reductive dehalogenation with 2N NaOH (6) or with $NaBH_4$ according to the method described by Egli (27).

6.4 Chromatography

6.4.1 Thin layer chromatography

The identification and separation of Iopamidol from its byproducts by thin layer chromatography is summarized in Table 16.

Table 16
TLC data for Iopamidol (28)

Solvent System	Rf_a	Rf_b
I	0.18	0.24
II	0.39	0.30
III	0.58	0.52
IV	0.26	0.30
V	0.28	0.21
VI	0.28	0.30
VII	0.43	0.47
VIII	0.31	0.30
IX	0.36	0.36
X	0.62	0.72
XI	0.45	0.45
XII	0.32	0.14

Support:

a) Silica gel 60 F_{254}, precoated plates, Merck
b) Cellulose F, precoated plates, Merck

Solvent System

I C
II C
III C
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6.4.2 High Pres

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- Inject

I	$CHCl_3$: MeOH : 25% NH_3	6:3:1 (v/v)
II	$CHCl_3$: MeOH : 25% NH_3	6:5:1 "
III	$CHCl_3$: MeOH : 25% NH_3	5:4:2 "
IV	$CH_3COOC_2H_5$: EtOH:25% NH_3	15:7:6 "
V	$CH_3COOC_2H_5$: iso-PrOH:25% NH_3	2:2:1 "
VI	iso-PrOH : 25% NH_3	4:1 "
VII	CH_3COCH_3 : iso-PrOH :25% NH_3	2:2:1 "
VIII	n-ButOH : MeOH : 25% NH_3	4:1:1 "
IX	sec-ButOH : iso-PrOH: 25% NH_3	5:2:3 "
X	$C_2H_5COCH_3$: n-PrOH: EtOH : 25% NH_3	10:1:2:7 "
XI	$C_2H_5COCH_3$: CH_3COOH : H_2O	15:3:5 "
XII	$CH_3COOC_2H_5$: CH_3COOH : H_2O	5:2:1 "

- Detection
  - Ultraviolet (254 nm)
  - 1% aqueous starch and subsequent exposure to UV light (254 nm) to give brown spots.

The best results in terms of separation of Iopamidol from its principal potential impurities were achieved using solvent system I with support a.

6.4.2 High Pressure Liquid Chromatography

The following HPLC method was developed for quali-quantitative determination of Iopamidol and of its by-products (29) :

- Apparatus : HPLChromatograph H.P.1084B with variable wavelength detector set at 240 nm
- Column : Lichrosorb RP18-5µm : 4x250 mm
- Injection : 20 µl

- Eluant A : water
- Eluant B : methanol 25% in water (v/v)
- Flow rate : 1.5 ml.min^{-1}
- Gradient profile :

min	%B	
0	7.5	
6	7.5	
18	35.0	
30	92.0	
34	92.0	
37	7.5	column recondi-
42	7.5	tioning

- Column temperature : 35°
- Retention time of Iopamidol : ~7.5 min

6.5 Analysis of impurities

A certain number of the most probable potential impurities arising from Iopamidol synthesis were characterized both chromatographically (TLC, HPLC) and structurally using spectroscopic methods, i.e. UV, IR, ^{1}H-NMR, FAB/MS (30).

6.5.1 Free aromatic amine

A manual method (6), based on the classic colorimetric reaction according to Bratton and Marshall, was set up for the determination of free aromatic amine; the procedure was automated for routine analysis both of the bulk product and of its injectable formulations (31).

6.5.2 Free iodine and free halides

Free iodine is detected by extraction with toluene of an acidic (H_2SO_4) aqueous solution of Iopamidol (2g/30 ml). Toluene must remain colorless (6).
Free halides are determined by addition of $AgNO_3$ to an acidic aqueous solution of Iopamidol and comparison of its turbidity with that of solutions containing a known amount of chlorides (6).

7. METABOLISM

7.1 Metabolism

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7. METABOLISM AND PHARMACOKINETICS

7.1 Metabolism

The metabolic fate of Iopamidol was studied in rabbits and dogs after intravenous and intrathecal administration (32, 33, 34, 35).
Both species excrete the compound unchanged, as demonstrated by thin layer chromatography of the urine and bile and by isolation from urine. Human studies (34, 36, 37) have shown that Iopamidol does not undergo any significant biotransformation or deiodination after intravenous as well as after intrathecal administration.

7.2 Pharmacokinetics

Several authors (32, 34, 35, 38, 39, 40, 41, 42) studied the pharmacokinetics of Iopamidol in various animal species both after intravenous and after intrathecal dosing.
When administered intravenously, the compound distributes to a volume equivalent to the extracellular fluid and is filtered almost exclusively by the kidneys. Also after intrathecal administration Iopamidol is eliminated rapidly through the kidneys.
The results of human studies (34, 36, 37, 43, 44, 45, 46, 47) are in general agreement with the data obtained in experimental animals. After intravenous injection the pharmacokinetics of Iopamidol is best described by an open linear, two-compartment model. The average plasma elimination half-life is about 2 hours. The volume of distribution is approximately equal to the extracellular fluid volume. Iopamidol is excreted predominantly through the renal route. Fecal elimination averages 1% or less of the dose, indicating minimal biliary excretion.
The distribution and elimination kinetics of Iopamidol after intrathecal injection was assessed by densitometric CT readings and by iodine assay of blood and urine (43).
CT readings were maximal at 1hr in the lumbosacral subarachnoid space and at 6hr in the cervical region.
Peak plasma levels were observed at 2.9 hr and were no longer detectable at 48 hr. The 48 hr urinary recovery averaged 66 ± 8%.
After oral administration to the rat, Iopamidol was

eliminated within 48 hr almost totally by the fecal route and only negligible amounts were found in urine (42).

7.3 Protein Binding

No binding of Iopamidol to plasma proteins and CSF proteins of dog and rabbit was observed (32). Human serum protein binding averaged less than 1% at 1hr postinjection (36).

8. DETERMINATION OF IOPAMIDOL IN BODY FLUIDS AND TISSUES

Most methods for assay of contrast media are based on the assumption that the amount of contrast medium in the sample is proportional to its iodine content.
This assumption is valid also for assay of Iopamidol in body fluids and tissues, since the injectable solutions contain only negligible amounts of I^- and no appreciable deiodination occurs in vivo (37).
A survey on methods for assay of iodinated contrast media has recently been published (49).
The following methods have been reported for assay of Iopamidol :

8.1 Colorimetry

A fully automated colorimetric method, originally developed for the determination of protein bound iodine (PBI) (50, 51), has been applied by several authors (33, 34, 36, 46, 60) for assay of Iopamidol in plasma, urine and CSF.

8.2 X-Ray Fluorescence

X-ray fluorescence analysis, developed for determination of iodine in vitro (48,52) and in vivo (52, 53, 61) was also utilized in several pharmacokinetic studies with Iopamidol (36, 43, 44, 54, 55, 62, 63, 64, 65).
Experimental details, precision and accuracy of X-ray fluorescence analysis of Iopamidol in body fluids and tissues by excitation of the $L\alpha$ line of iodine are reported by V.Lorusso et al. (55).

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8.3 Neutron activation

The high sensitivity of neutron activation analysis, which allows the measurement of iodine concentrations as low as 0.02 ppm, was used by O. Muratore et al.(40) for the study of iodine levels in different tissues of rats up to 62 days after injection of Iopamidol.

8.4 Radioactive labeling

Both ^{125}I- and ^{14}C-labeled Iopamidol have been employed for study of its pharmacokinetics and tissue distribution [Franchini ^{125}I (45), Kivisaari ^{125}I (56), Rosenbaum ^{14}C (39), Mc Kinstry ^{14}C (36)].

8.5 CT-Densitometry

Although less sensitive than the above mentioned methods, CT-densitometry, i.e. X-ray absorption measurements during computerized tomography, has been successfully utilized for evaluation of Iopamidol concentrations in vivo and in vitro (38, 57, 58).

8.6 High performance liquid chromatography [HPLC]

A specific and precise HPLC method for assay of Iopamidol in urine, plasma and CSF, applicable in the range 5-1000 mcg/ml for urine and 0.5-5000 mcg/ml for plasma and CSF has been reported by E.Felder et al. (59).
HPLC was also employed in the studies of D.Pitrè et al. (37) and D.Mc. Kinstry et al. (36).

References

1. E.Felder, D.Pitrè and P.Tirone, Il Farmaco, 32, 835 (1977).
2. D.Pitrè and E.Felder, Invest.Radiol. 15, S301 (1980).
3. E.Felder and D.Pitrè, U.S.Patent 4.001.323.
4. F.Bonati, E.Felder and P.Tirone, Invest.Radiol., 15, S310 (1980).
5. E.Felder, Invest.Radiol., 19, S168 (1984).
6. E.Felder, M.F.Zingales, and U.Tiepolo, Boll.Chim. Farmac., 120, 639 (1981).
7. S.Bradamante and G.Vittadini, Magn.Res.Chem., 25, 283 (1987).
8. A.Ripamonti, Bracco SpA, personal communication.
9. A.Clerici, P.Traldi, M.Grandi and D.Pitrè, Biomed. Mass Spectrom., 9, 257 (1982).
10. F.Ganazzoli, A.Albinati and D.Pitrè, Acta Cryst., C39 1570 (1983).
11. D.Pitrè and E.Felder, Invest.Radiol., 15, S301 (1980).
12. A.Albinati, Ist.Chim.Farmac.Univ.Milano, personal communication.
13. A.Liborio, Dipartim.Chim.Ind.Univ.Milano, personal communication.
14. Pregl-Roth, "Quantitative Organische Mikroanalyse", 7th ed., Springer Verlag, Wien, 1955, pg. 310.
15. M.Cabrini, Bracco SpA, personal communication.
16. E.Felder, D.Pitrè and M.Grandi, Il Farmaco,Pract.Ed., 37 3 (1982).
17. A.Albert, and E.P. Serjeant, "Ionization Constants of Acids and Bases", Methuen & Co.Ltd, London, 1962.
18. A.Leo, C.Hansch and D.Elkins, Chem.Rev., 71 525 (1971).
19. D.Scarcella, Bracco SpA, personal communication.
20. D.F.Burger, J.Phys.Chem., 67 2590 (1963).
21. B.Bördalen, H.Wang and H.Holtermann, Invest.Radiol., 5, 559 (1970).
22. W.H.Streng, H.E.Huber and J.T.Carstensen, J.Pharm. Sci., 67 384 (1970)
23. V.Lorusso, Bracco SpA, personal communication.
24. E.Mueller in " Methoden der Organischen Chemie ", Houben-Weyl, Band III, Teil 1, G.Thieme, Stuttgart, 1955, pg.343.
25. K.Thoma and G.Pfaff, Pharm.Ind., 37 552 (1975).
26. E.Felder, Invest.Radiol., 19 S164 (1984).
27. R.Egli, Z.Anal.Chem., 247 39 (1969)
28. M.Brocchetta, Bracco SpA, personal communication.
29. A.Pavilla, Bracco SpA, personal communication.
30. M.Grandi, D.P Lewis, Biomed
31. E.Felder, D.P (1975).
32. P.Tirone and
33. M.Kelly and K
34. D.Pitrè, P.Ti 35 827 (1980)
35. E.Felder, D.I 32 835 (1977)
36. D.N.Mc Kinsti Radiol., 19
37. D.Pitrè, M.F. 25 37 (1983).
38. J.Wilcox, C.A 359 (1986).
39. D.M.Rosenbaur 123 (1984).
40. O.Muratore, M.Sanzone, E
41. J.Wilcox, C.A Radiol., 18
42. N.Corsico, G at XXXII Co di Radiologi
43. K.L.Duchin, Amer.J.Neuro
44. B.P.Drayer, M.Ross,E.R.H S259 (1984).
45. D.Franchini, Clin.Pharm.R
46. P.Tirone, D.
47. A.J.Rommel, 15 421 (1980
48. A.A.Moss, L. 335 (1972).
49. K.Golman and Pharmacology 1984, pg 160
50. P.Hellstern, 4 241 (1980)
51. M.Riley and sium, New Yo
52. T.Groenberg, and S.Sjobe
53. L.Kaufman, I 167 (1973).

30. M.Grandi, D.Pitrè, A.Clerici and P.Traldi, I.A.S. Lewis, Biomed.Mass Spectrom., 10, 17 (1983).
31. E.Felder, D.Pitrè and M.Grandi, J.Pharm.Sci., 64 684 (1975).
32. P.Tirone and E.Ferrari, Ravs, 7 73 (1982).
33. M.Kelly and K.Golmann, Invest.Radiol., 16 159 (1981)
34. D.Pitrè, P.Tirone and G.Viviani, Il Farmaco Sci Ed., 35 827 (1980).
35. E.Felder, D.Pitrè and P.Tirone, Il Farmaco, Sci.Ed., 32 835 (1977).
36. D.N.Mc Kinstry, A.J.Rommel, and A.A.Superman, Invest. Radiol., 19 S171 (1984).
37. D.Pitrè, M.F.Zingales and C.Trevisan, Neuroradiology, 25 37 (1983).
38. J.Wilcox, C.A.Evill and M.R. Sage, Neuroradiology, 28 359 (1986).
39. D.M.Rosenbaum and W.J.H. Caldicott, Invest.Radiol.,19 123 (1984).
40. O.Muratore, S.Saitta, G.Mallarini, P.Corvisiero and M.Sanzone, Experientia, 39 119 (1983).
41. J.Wilcox, C.A.Evill, M.R.Sage and G.T.Benness,Invest. Radiol., 18 207 (1983).
42. N.Corsico, G.Malinverno and P.Tirone, paper presented at XXXII Congresso Nazionale "Incontri Mediterranei di Radiologia" SIRMN, Milano 15-20 June 1986.
43. K.L.Duchin, B.P.Drayer, M.Ross, S.Allen and M.Frantz, Amer.J.Neuroradiol., 7 895 (1986).
44. B.P.Drayer, S.Allen, C.Vassallo, U.Warner M.Bates, M.Ross,E.R.Heinz and D.S.Osborne, Invest.Radiol., 19 S259 (1984).
45. D.Franchini, N.Papa, G.Ugolotti and M.Rinetti. Int.J. Clin.Pharm.Res., 2 343 (1982).
46. P.Tirone, D.Ferrari and G.Viviani, Ravs, 7 83 (1982).
47. A.J.Rommel, S.Singhvi and A.A.Sugerman,Invest.Radiol. 15 421 (1980).
48. A.A.Moss, L.Kaufman and J.A.Nelson, Invest.Radiol., 7 335 (1972).
49. K.Golman and T.Almen "Handbook of Experimental Pharmacology", Vol.73, M.Sovak Ed., Springer Verlag, 1984, pg 160.
50. P.Hellstern, H.E.Keller and B Weinheimer, J.Mol.Med., 4 241 (1980).
51. M.Riley and N.Gochman, Technicon International Symposium, New York, 1964.
52 T.Groenberg, T.Almen, K.Golman, K.Liden, S.Mattsson and S.Sjoberg, Phys.Med.Biol., 26 501 (1981).
53. L.Kaufman, D.Shames, and M.Powell, Invest.Radiol., 8 167 (1973).

54. M.Thompson, W.L.Foster, R.A.H.Halvorsen, N.R.Dunnick, A.J.Rommel and M.Bates, Amer.J.Roentgenol.,142 329 (1984).
55. V.Lorusso, P.Tirone and E.Felder, paper presented at III Convegno "Applicazioni industriali delle tecniche a raggi-X, difrattometria e fluorescenza", Bressanone (I), 18-20 marzo 1986.
56. L.Kivisaari, P.B.Dean and M.Kormano, Contrast Media in Radiology, Proc.Lyon, 1981 M.Auriel Ed., Springer Berlin, pg 324.
57. M.R.Sage and J.Wilcox, AJNR, 4 1181 (1983).
58. R.E.Brennan, S.Rapoport, I.Weinberg, H.M.Pollack and J.A.Curtis, Invest.Radiol., 17 95 (1982).
59. E.Felder, A.Gallotti and A.Pavilla, Poster presented at 2nd International Symposium on Drug Analysis, Brussels, 27-30 May 1986.
60. O.P. Eldevik, V.M. Haughton and E.A. Sasse, Invest.Radiol., 15 S260 (1980).
61. D.E. Leiden and W.K. Nonider, CRC Crit.Rev.Clin.Lab.Sci., 7 393 (1977).
62. S.Sjoberg, T.Almen and K.Goldman, Acta Radiol.Diagn.Suppl., 362, 93 (1980).
63. R.F.Spataro, H.W.Fischer, and L.Boylan, Invest.Radiol., 17, 494 (1982).
64. F.A.Burgener and O.H. Gutierrez, Invest.Radiol., 20, 399 (1985).
65. F.A. Burgener and S.D. Steinmetz, Invest.Radiol., 20, 626 (1985).

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1: Invest Radiol. 1996 Jun;31(6):338-44.

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Neurotolerability of nonionic X-ray contrast media. The role of chemotoxicity.

Luzzani F, Morisetti A, Bussi S, Tirone P, de Haen C.

Milano Research Centre, Bracco Spa, Italy.

RATIONALE AND OBJECTIVES: Because small quantities of x-ray contrast agents can cross the blood-brain barrier, the authors evaluate the properties that contribute to neurotoxicity. METHODS: The acute toxicity of various monomer and dimer contrast media was assessed after intracerebroventricular (ICV) injection to mice and intracisternal (ICI) injection to rats. RESULTS: In mice, median lethal dose (LD50) values for monomer contrast media apart from iohexol were higher than those for dimer contrast media. In rats, iopentol and iopromide were more neurotoxic than all other contrast media. The signs of toxicity for all contrast media included convulsions, dyspnea, hypoactivity, and sedation. Hypertonic D-mannitol solution was tolerated as well as artificial cerebrospinal fluid. Neither the hydrophilicity of the molecules nor the physicochemical properties of their solutions explain the toxicities satisfactorily. CONCLUSIONS: Neurotoxicity of monomer or dimer contrast media depends more on chemical structure characteristics other than hydrophilicity than on the physicochemical characteristics of their solutions.

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Fast Atom Bombardment Mass Spectrometry of By-products in the Iopamidol Synthesis

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The fast atom bombardment mass spectra of by-products in the synthesis of Iopamidol are reported and their mass spectrometric fragmentation processes are discussed. This technique represents the ionization method which gives the best structural information on Iopamidol and similar compounds.

INTRODUCTION

Iopamidol, (S)-*N*-*N'*-bis-(2-hydroxy-1-(hydroxymethyl)-ethyl)-5-[(2-hydroxy-1-oxypropyl)-amino]-2-4-6-triiodo-1,3-benzenedicarboxamide,[10] a new non-ionic water soluble contrast medium, is widely used in angiography[1-3] and mielography.[4-9] During studies of its synthesis, carried out under different experimental conditions, several by-products, compounds 2, 4, 5 and 7–9, have been isolated.

In a previous paper[12] the mass spectral behaviour of Iopamidol and its derivative using different ionization methods was described and the spectral data obtained were found to be mutually complementary. Conclusive information was obtained by using the fast atom bombardment (FAB) technique.[13] In this paper the same method is extended to the above mentioned compounds; their FAB spectra are reported and their fragmentation patterns discussed. Identification of these products was confirmed by synthesis as described in the experimental section.

EXPERIMENTAL

Apparatus and methods

FAB mass spectra of compounds 2, 4, 5 and 7–9 were obtained with a VG Micromass 70–70 E by mixing the samples with glycerol.

Thin layer chromatographic analyses were carried out on silica gel plates (5 × 20 cm, 60 F_{254} precoated, Merck) by using the following systems as a developing solvent: (A) $CHCl_3:CH_3OH:NH_3$ 25% = 6:3:1; (B) $CH_3COCH_3:C_2H_5OH:NH_3$ 25% = 2:1:1; (C) $CH_3COC_2H_5:CH_3COOH:H_2O$ = 15:3:5. High-performance liquid chromatographic analyses were carried out on a Hewlett–Packard 1084-B instrument equipped ith Hibar RT columns packed with Lichrosorb RP-18, 5 μm, Merck. The eluants were composed of water (Solvent A) and 25% (v/v) acetonitrile/water (Solvent B). A flow rate of 90 ml h^{-1} and the following gradient profile were used:

Time (min)	%B	
6	7.5	
18	35	
30	92	
34.5	92	stop

^1H NMR spectra were recorded on a Varian XL-100 instrument operating at 100 MHz. Samples were dissolved in DMSO-$^2H_6$. UV spectra were obtained by a Cary 219 spectrophotometer. IR spectra were taken by using a Perkin–Elmer 257 spectrometer in KBr pellets.

Synthesis

The general scheme of synthesis (Scheme 1) for the preparation of the by-products of Iopamidol is reported. The starting material is 5-amino-2-4-6-triiodoisophthalic acid dichloride (1) which reacts with 1,3-dihydroxy-2-amino-propane to give product 2. In order to obtain the other compounds, the dichloride (1) is transformed into the corresponding acylaminochoride (3) from which, through a reaction with 1,3-dihydroxy-2-aminopropane and according to the acylamino group present, Iopamidol and products 4 and 5 are obtained.

Treating the chioride (3c) with 1 mol of 1,3-dihydroxy-2-aminopropane yields the hemichloride (6) which is further transformed into the asymmetric amides 7 and 9 and the acid 8. The conditions used for these transformations are similar to those reported in earlier studies.[11-14]

Chromatographic and spectroscopic data

N,N'-bis-[(1,3-dihydroxy-2-propyl)-5-amino]-2-4-6-triiodoisophthaldiamide (2). m.p., 259–260 °C dec. (from water); TLC, R_f 0.25 (A); HPLC, t_R = 12.5 min. UV,

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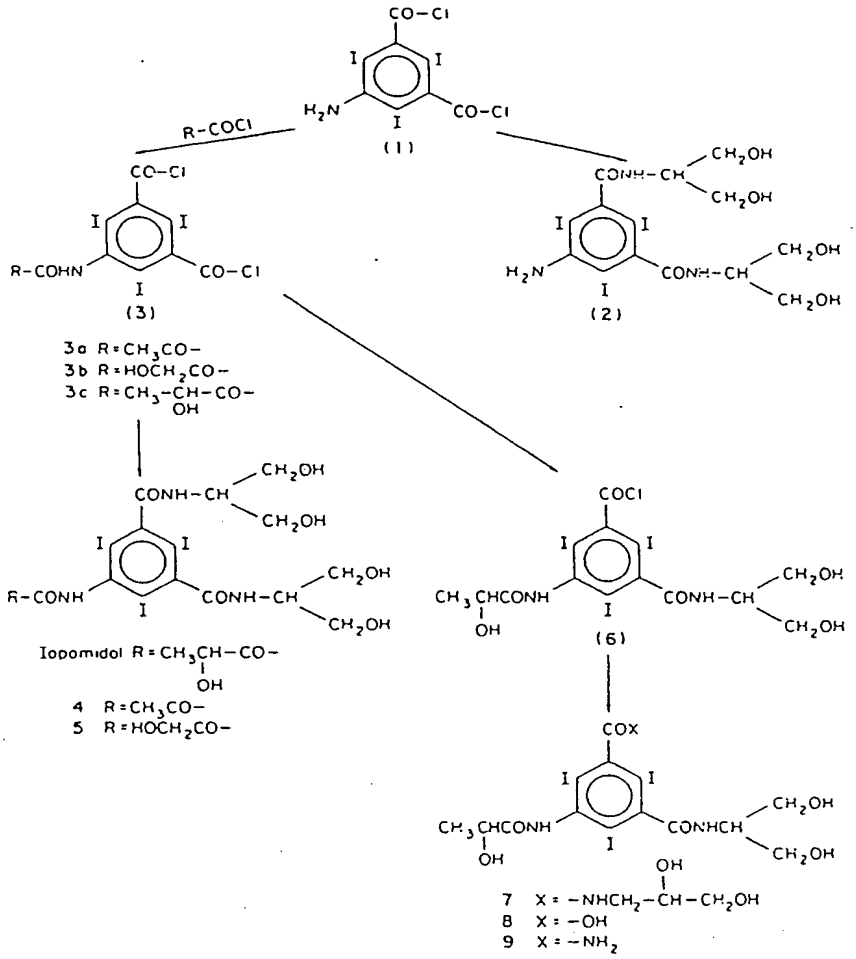
CCC–0306–042X/83/0010–0017$03.50



Scheme 1. Synthetic routes to by-products in iopamidol synthesis.

231.5 nm (ε = 30,500), 319.5 (ε = 5200) in MeOH. IR 3415 and 3325 (ν_{as}, ν_s NH_2), 3380 (νOH) 3285 and 3060 NH, amide), 1630 (νC=O, amide) 1550 (δNH + νCN, mide), 1070 and 1050 cm^{-1} (νC—O aclohol). ¹H NMR: δ_H (ppm) 3.4–4.0 (10H, $4CH_2O$ + CH—N,m); 4.4 (4H, 4OH, exch., m); 5.4 (2H, NH_2, s, broad); 7.45 (1H, CO—NH, d); 8.0 (1H, CO—NH, d).

***N,N'*-bis[(1,3-dihydroxy-2-propyl)-5-acetamido]2-4-6-triiodoisophthaldiamide (4).** m.p., 286–288 °C dec. (from CH_3OH); TLC, $R_f = 0.19$ (A); $R_f = 0.38$ (B); HPLC, $t_R = 9.0$ min. UV, 241 nm (ε 29, 500), CH_3OH. IR, λ_{max} 3380 (νOH); 3250 (νNH, amide); 1635 (νC=O, amide); 1545 (δNH and νCN, amide); 1045 (νC—O, alcohol). ¹H NMR, δ_H (ppm) 2.0 (3H, CH_3CO, s); 3.0–4.0 (10H, $4CH_2$—O + 2CH—N, m); 4.44 (4H, 4OH, exch., s, broad); 7.54 and 8.15 (2H, Ph–CONH, s, broad); 9.8 (1H, Ph—NH—CO, s, large).

***N,N'*-bis(1,3-dihydroxypropyl)-5-hydroxyacetamido-2-4-6-triiodoisophthaldiamide (5).** TLC, $R_f = 0.38$ (C) (UV); HPLC, $t_R = 4.4$ min. UV, 241 nm (ε 29,250), CH_3OH. IR, λ_{max} 3390 (νOH); 3240 (νNH, amide); 1645 C=O, amide); 1535 (δNH and νCN, amide); 1050 cm^{-1} (νC—O, alcohol). ¹H NMR, δ_H (ppm) 3.2–3.9 (11H, $4CH_2O$ + 2CH—N + 1 OH, m); 3.99 (2H, CH_2CO, s); 4.48 (4H, 4OH, s, exch); 7.54 and 8.16 (2H, Ph—COHN—); 9.70 (1H, Ph—NHCO, s).

***S*(−)-*N*-(2,3-dihydroxy-1-propyl)-*N'*-(1,3-dihydroxy-2-propyl)-5-[(2-hydroxy-1-oxypropyl)-amino]-2-4-6-triiodoisophthal-diamide (7).** TLC, $R_f = 0.25$ (A); $R_f = 0.65$ (C); HPLC, $t_R = 8.5$ min. UV, 242 nm (ε 29,000) in water. IR, 3360 (νOH); 3260 and 3080 (νNH, amide); 1650 (νCO, amide); 1550 (δNH + νCH, amide); 1120 (νCO sec.-alcohol); 1045 (νCO p-alcohol). ¹H NMR, δH (ppm) 1.4 (3H, CH_3, d); 3.1–3.8 (10H, $3CH_2$—O + CH—N+CH—C, m); 4.09 (1H CH—OH, q); 4.47 (3H, 3OH s, exch); 4.65 (1H, OH, exch); 5.62 (1H, OH, t, exch); 7.66–8.45 (2H, 2 Ph—CO—NH, m, broad); 9.96 (1H, Ph—NH—CO, s).

***S*(−)-*N*-(1,3-dihydroxy-2-propyl)-5-[(2-hydroxy-1-oxypropyl)-amino]-2-4-6-triiodoisophthalamic acid (8).** m.p., 285 °C, (dec); TLC, $R_f = 0.07$ (A); $R_f = 0.27$ (C); HPLC, $t_R = 0.9$ min. UV, 242 nm (ε = 30,000 in MeOH). IR, 3340 (νOH); 3260 (νNH, amide); 2500 (νOH, acid); 1678 (νCO, acid); 1650 (νCO, amide); 1518 (δNH + νCH, amide); 1135 (νC—O sec.-alcohol); 1038 cm^{-1} (νC—O *p*-alcohol). ¹H NMR, δH (ppm) 1.39 (3H, CH_3, d); 3.5–6.5 (4H, 3OH—COOH, broad, exch); 3.60 (4H, $3CH_2$—O, m); 3.80 (1H, CH—N, t);

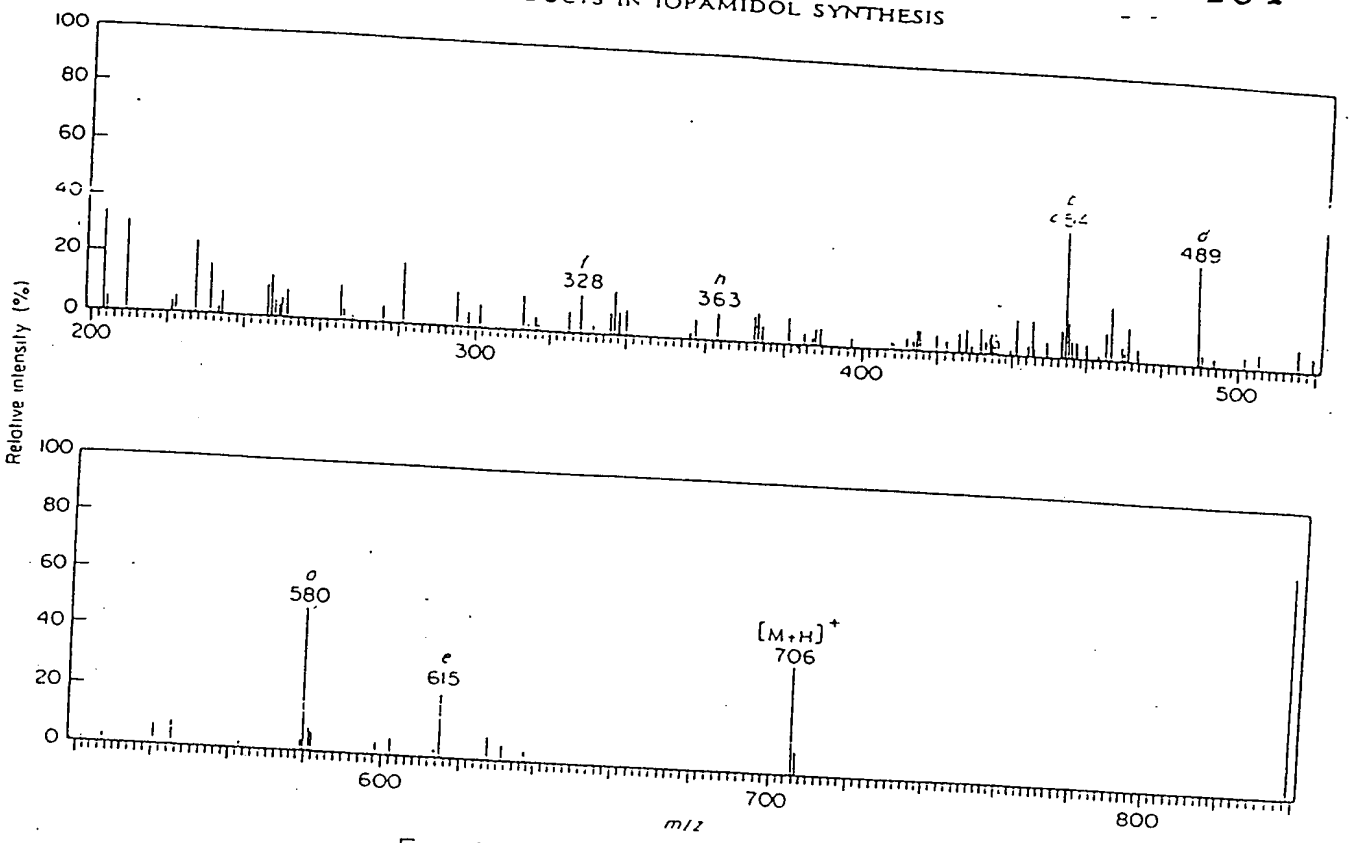


Figure 1. FAB mass spectrum of compound 2.

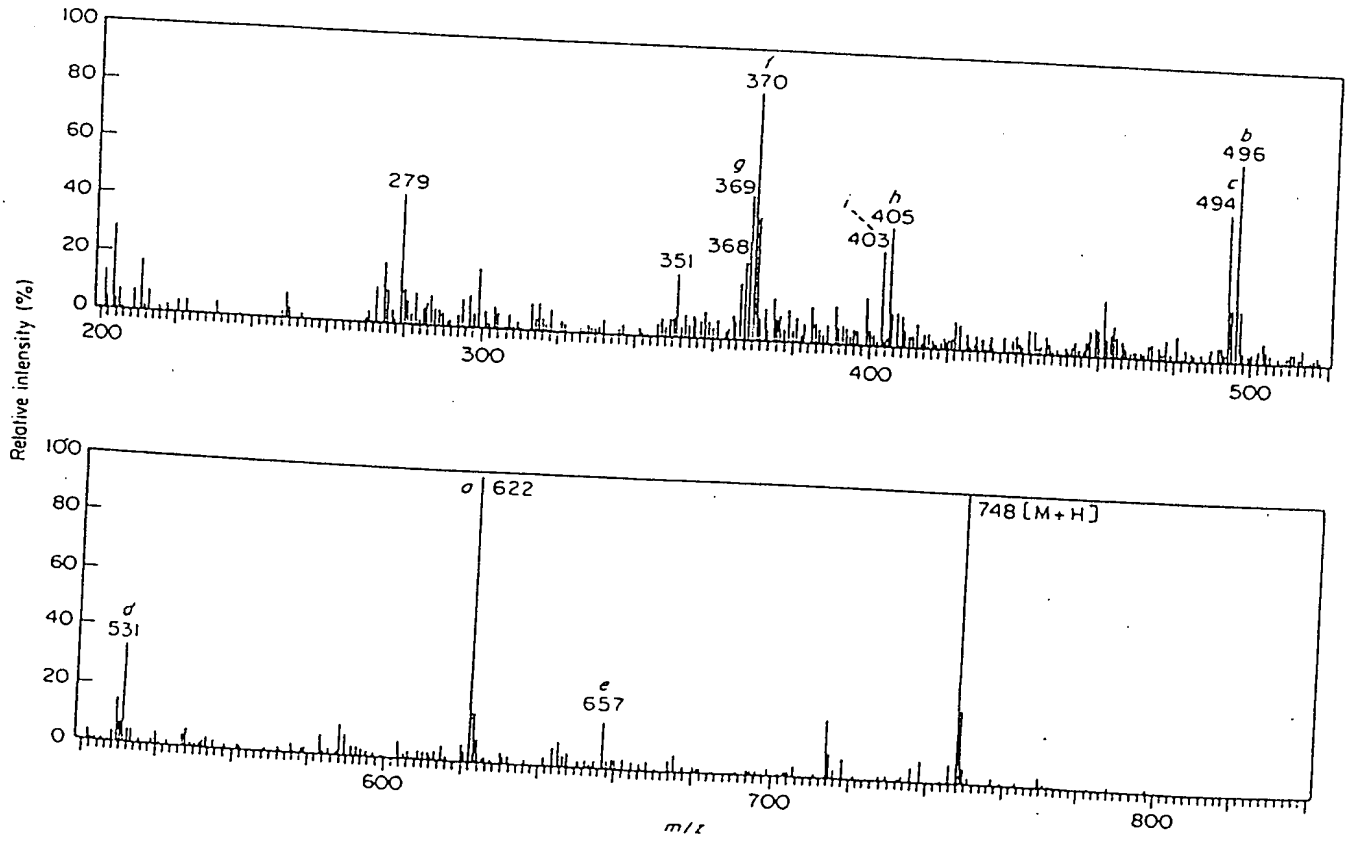


Figure 2. FAB mass spectrum of compound 4.

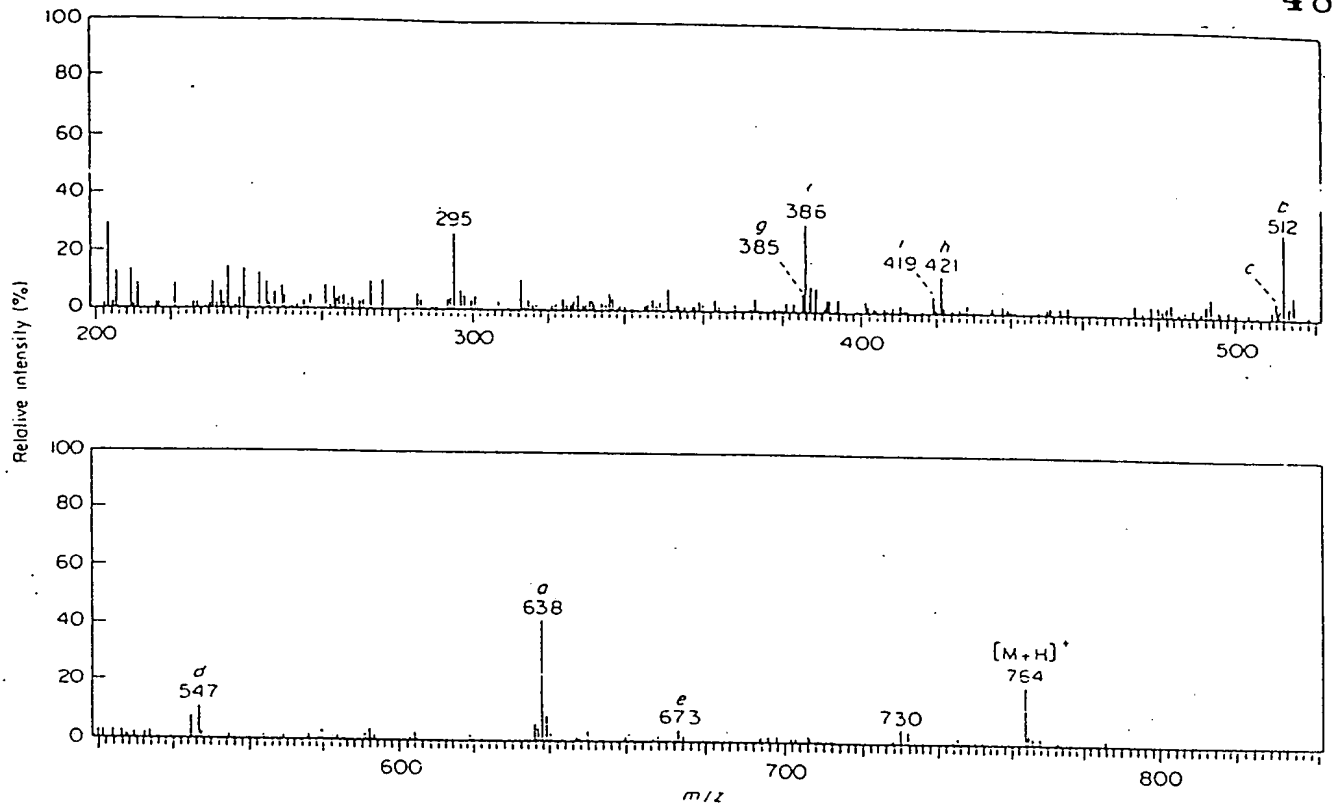


Figure 3. FAB mass spectrum of compound 5.

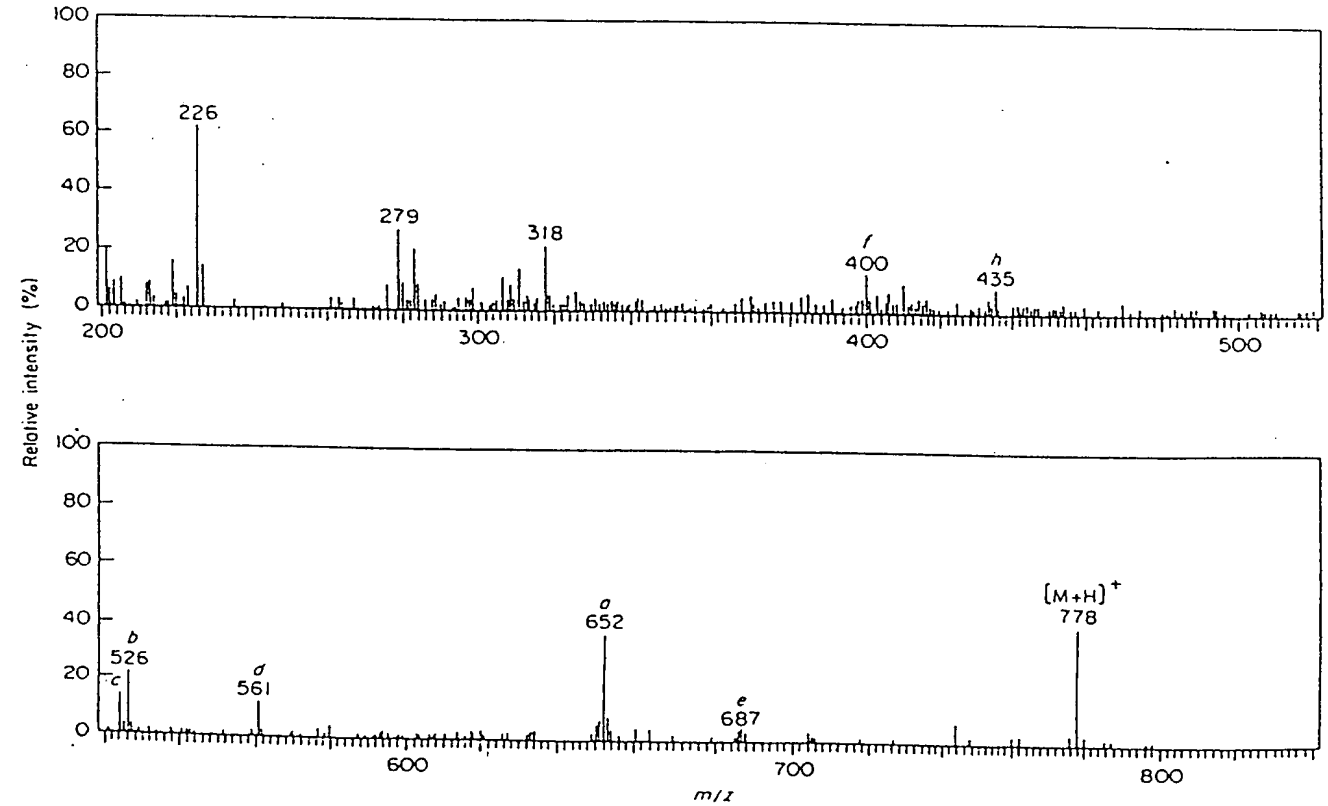


Figure 4. FAB mass spectrum of compound 7.

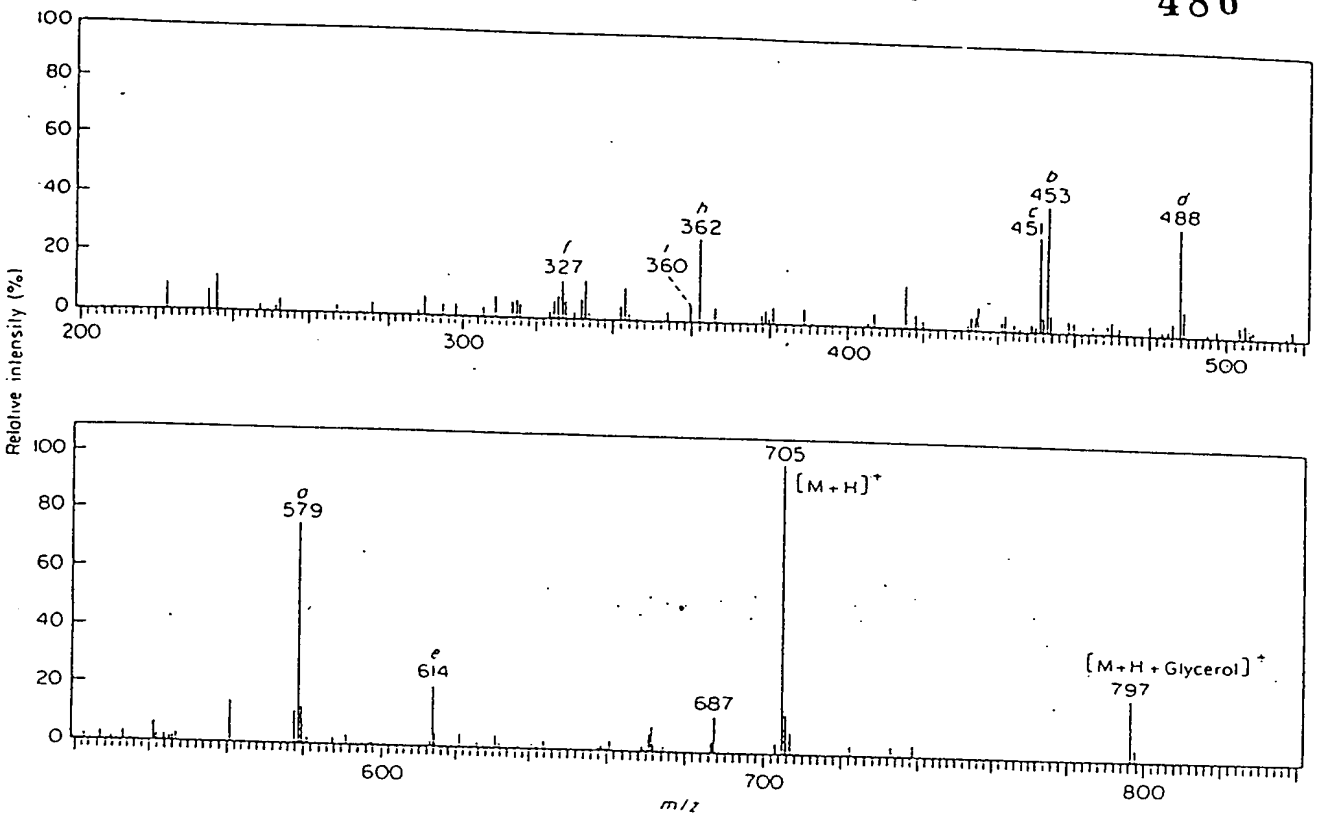


Figure 5. FAB mass spectrum of compound 8.

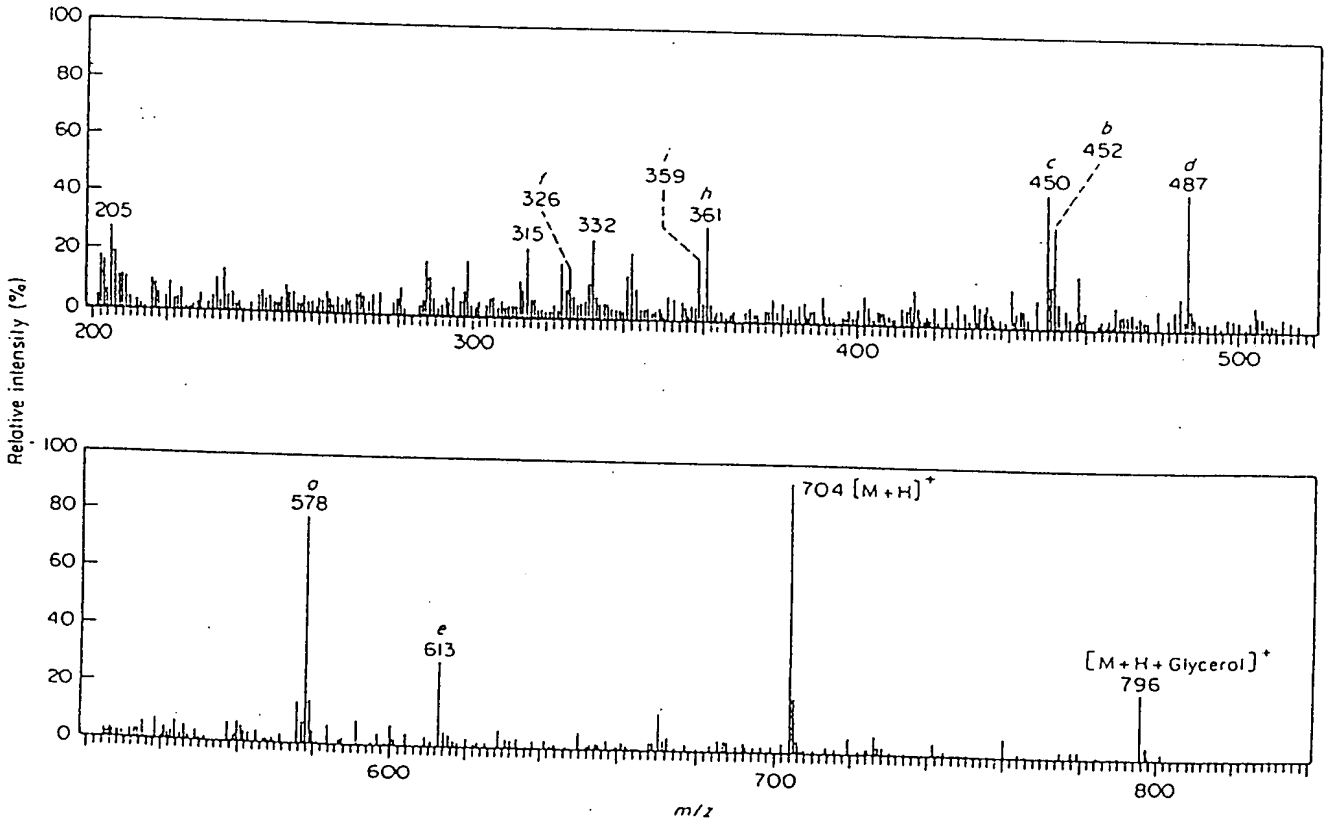
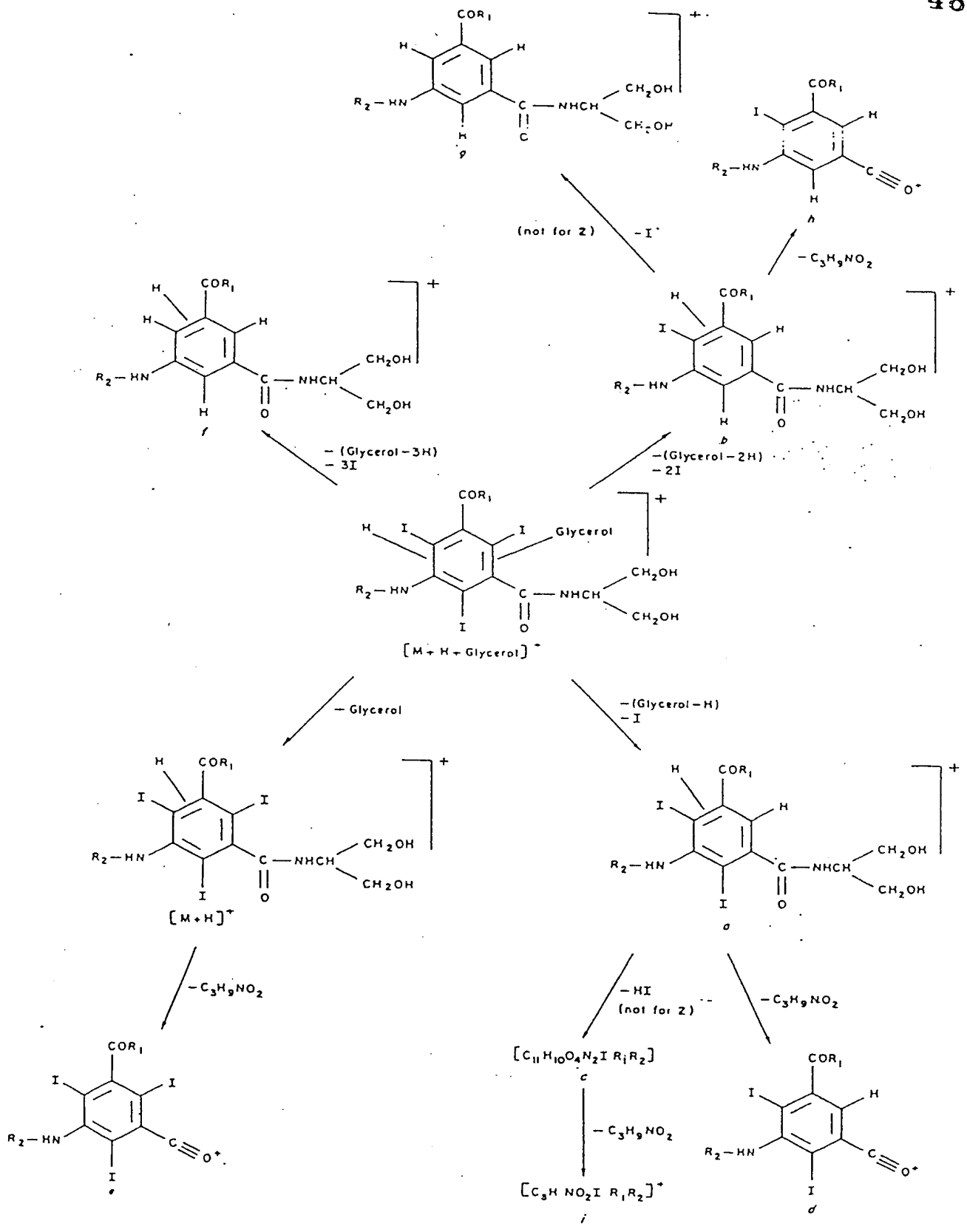


Figure 6. FAB mass spectrum of compound 9.



Scheme 2. FAB Mass spectral fragmentation behaviour of byproducts in iopamidol synthesis.

1.15 (1H, CH—, q); 8.18 (1H, Ph—CO—NH, d); 9.64 (1H, Ph—NH—CO, s).

N-(1,3-dihydroxy-2-propyl)-5-[(2-hydroxy-1-oxypropyl)-amino]-2-4-6-triiodoisophthalamide (9). m.p., 115–118 °C; TLC, $R_f = 0.24$ (A); HPLC, $t_R = 6.1$ min. UV, 242 nm ($\varepsilon = 27{,}200$) in water. IR, 3320; 3180; 3070 (νOH + νNH); 1670 (νCO, amide); 1530 (δNH + νCN, amide); 1120 (νC—O, sec.-alcohol); 1045 (νC—O, *p*-alcohol) cm^{-1}. ^1H NMR, δH (ppm) 1.38 (3H, CH_3—, d); 3.40–4.0 (5H, $2CH_2O + CH—N$, m); 4.18 (1H, CH—O, q); 4.45 (2H, CH_2—OH, t); 5.60 (1H, CH—OH, s, broad, exch); 7.62 (2H, Ph—$CONH_2$, s, broad); 7.97 and 8.83 (1H, Ph—CO—NH, d); 9.70 (1H, Ph—NH—CO, s, broad).

RESULTS AND DISCUSSION

The FAB mass spectra of compounds 2, 4, 5 and 7–9 are reported in Figs 1–6. As can be seen, protonated molecular ions are always present, while the $[M+H+Glycerol]^+$ species, already found in the FAB mass spectrum of Iopamidol,[12] are present in the spectra of compounds 8 and 9 only.

Very abundant ions are present corresponding to $[M+2H-I]^+$ (*a*), $[M+3H-2I]^+$ (*b*) and $[M+4H-3I]^+$ (*f*). Ions of this kind are commonly observed in CI (NH_3) mass spectrometry and this substitution reaction may therefore be due to ion molecule reaction in gas phase as well as in solution. This surprising behaviour, surely not due to sample impurities, may well be explained by the following equation:

$$M + \text{Glycerol} \rightleftharpoons [\text{M-complex}]\ \text{Glycerol}$$

$$\text{FAB} \rightarrow \begin{cases} [M+H]^+ \\ [M+2H-I]^+ \\ [M+3H-2I]^+ \\ [M+4H-3I]^+ \\ [M+H+\text{glycerol}]^+ \end{cases}$$

i.e. these deiodinated and hydrogenated species may originate from a complex formation between glycerol and the compounds under study which, in FAB conditions, gives rise to the species described above. The absence of $[M+H+glycerol]^+$ ions in the mass spectra of compounds 2, 4, 5 and 7 may be due either to the lack of their formation or to their decomposition kinetics being faster than those of the $[M+H+glycerol]^+$ ions of compounds 8 and 9. We are inclined to the second hypothesis, which is well supported by the presence of the other deiodinated and hydrogenated species. Comparing the FAB spectra of compounds 2, 4, 5 and 7–9 the common fragmentation pattern reported in Scheme 2 has been obtained.

In addition to formation of ions *a*, *b* and *f*, losses of $C_3H_9NO_2$, corresponding to CO—NH bond cleavage with H rearrangement, are also present (ions *a*, *i*, *e* and *h*).

Other minor decomposition processes absent in the fragmentation pattern of compound 2 only, are due to loss of I˙ from ions *b* (ions *g*) and HI loss from ions *a* (ions *c*).

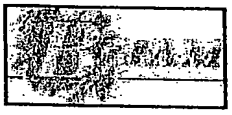
Acknowledgements

The authors wish to thank the referees of this paper for their useful suggestions.

REFERENCES

1. B Damascelli, M. Gasparini, P. L. Barigozzi, F. Fossati-Bellani, F. Garbagnati, A. Prada and E. Ceglia, *Clin. Radiol.* 31, 61 (1980).
2. E. W. Fletcher, *Br. J. Radiol.* 55, 36 (1982).
3. J. B. Partridge, P. J. Robinson, C. M. Turnbull, J. B. Stoker, R. M. Boyle and G. W. Morrison, *Clin. Radiol.* 32, 451 (1981).
4. M. Leonardi, G. Fabtis, T. Penco, C. Ceccotto and E. Biasizzo, *J. Neuroradiol.* 8, 55 (1981).
5. G. Belloni, G. Bonaldi, L. Moschini and N. Quilici, *Neuroradiology* 21, 97 (1981).
6. Hammer, *Radiologe* 21, 274 (1981).
7. B. Hammer and W. Lackner, *Neuroradiology* 19, 119 (1980).
8. E. Signorini, E. Ciorba, G. P. Pelliccioli, N. Caputo and G. L. Piccinin, *Rays* 4, 47 (1979).
9. G. Nuzzo, C. Malaguti, L. Mavilla, L. Fagioli and C. Trevisan, *Radiol. Med.* 66, 211 (1980).
10. INN, *Chronique OMS* No. 9, liste 40 (1978).
11. E. Felder and D. Pitrè, US Patent 4 001 323.
12. A. Clerici, P. Traldi, M. Grandi and D. Pitrè *Biomed. Mass Spectrom.* 9, 257 (1982).
13. M. Barber, R. S. Bordoli, R. O. Sedgwick and A. N. Tyler, *J. Chem. Soc. Chem. Commun.* 325 (1981).
14. D. Pitrè and E. Felder, *Invest. Radiol.* 15, 301 (1980).

Received 26 May 1982



IOPAMIDOL

Introduction dans BIAM : 18/2/1992
Dernière mise à jour : 18/6/1999
Etat : validée

- Identification de la substance
- Propriétés Pharmacologiques
- Mécanismes d'action
- Effets Recherchés
- Indications thérapeutiques
- Effets secondaires
- Effets sur la descendance
- Pharmaco-Dépendance
- Précautions d'emploi
- Contre-Indications
- Posologie & mode d'administration
- Pharmaco-Cinétique
- Bibliographie
- Spécialités contenant la substance

Identification de la substance

Formule Chimique :
N,N'-BIS(HYDROXY-2 HYDROXYMETHYL-1 ETHYL) TRIIODO-2,4,6 LACTAMIDO-5 ISOPHTALAMIDE.

Ensemble des dénominations

BAN : IOPAMIDOL
CAS : 62883-00-5
DCF : IOPAMIDOL
DCIR : IOPAMIDOL
USAN : IOPAMIDOL
bordereau : 2728
code expérimentation : B-15000
code expérimentation : SQ-13396
dci : iopamidol
rINN : IOPAMIDOL

Classes Chimiques

- IODE DERIVE
- ISOPHTALAMIDE

Proprietés Pharmacologiques

1. PRODUIT DE CONTRASTE (*principale certaine*)
   Produit de contraste monomère triiodé non ionique.

2. PRODUIT DE CONTRASTE IODE (*principale certaine*)
   Produit de contraste triiodé non ionique de basse osmolarité.

Mécanismes d'action

1. *principal*
   Produit de contraste iodée non ionique.
   L'absence de dissociation de la molécule serait responsable d'une réduction des effets indésirables

Effets Recherchés

1. PRODUIT DE CONTRASTE (*principal*)

Indications Thérapeutiques

1. ANGIOGRAPHIE (*principale*)
2. ANGIOCARDIOGRAPHIE (*principale*)
3. ARTERIOGRAPHIE (*principale*)
4. CORONAROGRAPHIE (*principale*)
5. UROGRAPHIE INTRAVEINEUSE (*principale*)
6. HYSTEROSALPINGOGRAPHIE (*principale*)
7. SACCORADICULOGRAPHIE (*principale*)
8. MYELOGRAPHIE (*principale*)
9. CISTERNOGRAPHIE (*principale*)

Effets secondaires

1. SENSATION DE CHALEUR (*CERTAIN RARE*)
2. TOUX (*CERTAIN RARE*)
3. CEPHALEE (*CERTAIN*)
4. RASH (*CERTAIN RARE*)
5. URTICAIRE (*CERTAIN RARE*)

6. PRURIT (*CERTAIN RARE*)

7. OEDEME DES PAUPIERES (*CERTAIN TRES RARE*)

8. NAUSEE (*CERTAIN TRES RARE*)
   Signe annonçant un accident sévère.

9. VOMISSEMENT (*CERTAIN TRES RARE*)
   Signe annonçant un accident sévère.

10. OEDEME ANGIONEUROTIQUE (*CERTAIN TRES RARE*)

11. BRONCHOSPASME (*CERTAIN TRES RARE*)

12. CRISE CONVULSIVE (*CERTAIN TRES RARE*)

13. COLLAPSUS CARDIOVASCULAIRE (*CERTAIN TRES RARE*)

14. ARRET CARDIAQUE (*CERTAIN TRES RARE*)

15. ARYTHMIE (*CERTAIN TRES RARE*)

16. SYNDROME MENINGE (*CERTAIN*)
    Condition(s) Exclusive(s) :
    VOIE INTRATHECALE

17. INSUFFISANCE RENALE(AGGRAVATION) (*CERTAIN*)
    Condition(s) Exclusive(s) :
    DIABETIQUE

    A la suite d'une coronarographie :
    - Am J Med 1991;89:615-620.

Effets sur la descendance

1. INFORMATION MANQUANTE DANS L'ESPECE HUMAINE

2. NON TERATOGENE CHEZ L'ANIMAL
   Etude chez le rat, le lapin.

Pharmaco-Dépendance

1. NON

Précautions d'emploi

1. GROSSESSE

2. ANTECEDENTS ALLERGIQUES

A utiliser de préférence à l'ioxaglate au cours de l'angiographie cardiaque chez des sujets à antécédents allergiques. La fréquence des accidents de type allergique serait 3.4 fois moindre avec l'iopamidol qu'avec l'ioxaglate :
- J Am Coll Cardiol 1992;19:899-906.

3. ALLERGIE A L'IODE

4. INSUFFISANCE RENALE

Contre-Indications

1. ALLAITEMENT

Posologie et mode d'administration

- En angiographie:
Les doses moyennes à employer sont variables selon le type d'examen.
- En urographie:
Les doses doivent être adaptées au poids et à la fonction rénale du sujet qui doit être à jeun sans restriction hydrique.
- En neuroradiologie:
Les doses doivent être adaptées à la région explorée et à la technique choisie.Le produit sera à injecter lentement.

Pharmaco-Cinétique

- 1 - DEMI VIE 2 heure(s)
- 2 - ELIMINATION voie rénale

Répartition
Liaison aux protéines plasmatiques négligeable (1%).
Demi-Vie
2 h.
Métabolisme
Non métabolisé.
Elimination
Voie rénale:
30 et 80% de la dose injectée sont éliminés dans les urines respectivement en 1 et 8 h.

Bibliographie

- J Neurosurg 1991;74:60-63. (EFFETS SECONDAIRES)* réactions allergiques.

Spécialités

Pour rechercher les spécialités contenant cette substance, consultez le site www.vidal.fr

Principe actif présent en constituant unique dans les spécialités étrangères suivantes :

Attention ! Données en date de janvier 2000.

- IOPAMIRON (ALLEMAGNE)

Retour à la page d'accueil

La Radiologia Medica - Radiol Med 107 (Suppl 1 al N. 4): 8-31, 2004
Edizioni Minerva Medica - Torino

Fondamenti sui mezzi di contrasto iodati e reazioni avverse

G. P. FELTRIN - M. ZANDONÀ - V. BORILE - C. RETTORE - D. MIOTTO

Introduzione

Lo sviluppo del mezzo di contrasto (MdC) è iniziato poco tempo dopo la scoperta dei raggi X, non appena ci si rese conto che la maggior parte delle strutture del corpo umano risultano invisibili e pertanto non documentabili a causa della scarsa radiopacità. Ma l'analoga necessità si è ripresentata con l'introduzione di altri principi fisici ed altre metodiche diagnostiche, quali l'Ultrasonografia e la Risonanza Magnetica.

L'impiego dei mezzi di contrasto è stato guidato dalla necessità della evidenziazione anatomica dell'apparato da studiare, dalla sua funzione e dalla via di somministrazione del mezzo stesso. Molto presto l'attenzione si pose su due elementi che rispondevano bene alla necessità del contrasto ai raggi X: il bario nella forma di solfato e lo iodio. Quest'ultimo fu inizialmente somministrato per via endovenosa come sale, ioduro sodico Ioduron®, con risultati non molto dissimili da quelli ottenuti con i MdC realizzati successivamente. Differivano in termini di concentrazioni, dose utilizzata e tollerabilità.

Nelle immagini radiologiche la duplice esaltazione di contrasto delle componenti anatomiche e funzionali di un apparato è quasi sempre indissolubile, poichè la somministrazione sfrutta la funzione specifica dell'apparato stesso, così che la presenza, la concentrazione e la scomparsa dello stesso mezzo di contrasto finisce per dare informazioni sul fenomeno funzionale (ad esempio la vascolarizzazione di un organo o l'escrezione del mezzo).

Particolarmente con le nuove tecniche d'immagine, sia mediante radiazioni X che segnali di campi magnetici che ultrasuoni, il principio del mezzo di contrasto ha notevolmente esaltato la potenzialità diagnostica e la qualità delle indagini, sia per le informazioni anatomiche che per quelle funzionali.

Visualizzazioni anatomo-morfologiche. Realizzando un incremento di contrasto si ottengono:

— variazioni di concentrazione nei diversi tessuti;

— variazioni temporali dell' opacità o dell'intensità di segnale degli stessi tessuti;

— variazioni della qualità del segnale del MdC e della separazione tra diversi componenti dei tessuti.

Visualizzazione di funzioni:

— escrezione-eliminazione renale, biliare;

— processi di trasporto: circolazione ematica, urinaria, biliare, contenuto intestinale;

— diffusione, fissazione (enhancement), scomparsa (wash-out), barriere tissutali ed emato-encefalica;

— metabolismo (RM).

Talora le informazioni ottenute sono strettamente legate e correlate, tali da rendere virtuale la separazione tra dati morfologici-anatomici e funzionali.

Classificazione dei mezzi di contrasto radiografici

Il contrasto negli esami radiologici è generato dall'assorbimento dei raggi X operato dal mezzo presente lungo il decorso del fascio radiante. L'assorbimento dipende dal numero atomico e dalla concentrazione del MdC. La differenza dell'assorbimento (contrasto) è la genesi dell'immagine per determinati organi o tessuti naturalmente provvisti di contrasto (osso, aria, polmone) o raggiunti da quello introdotto, che li rende visualizzabili.

L'introduzione di sostanze a bassa densità come aria o anidride carbonica rende le strutture contenenti o circostanti visibili per la riduzione di assorbimento indotta: queste sostanze si chiamano MdC negativi. Le sostanze che invece contengono elementi ad elevato numero atomico come bario o iodio aumentano l'assorbimento delle radiazioni e si chiamano MdC positivi. Essi sono sintetizzati nella Tabella I.

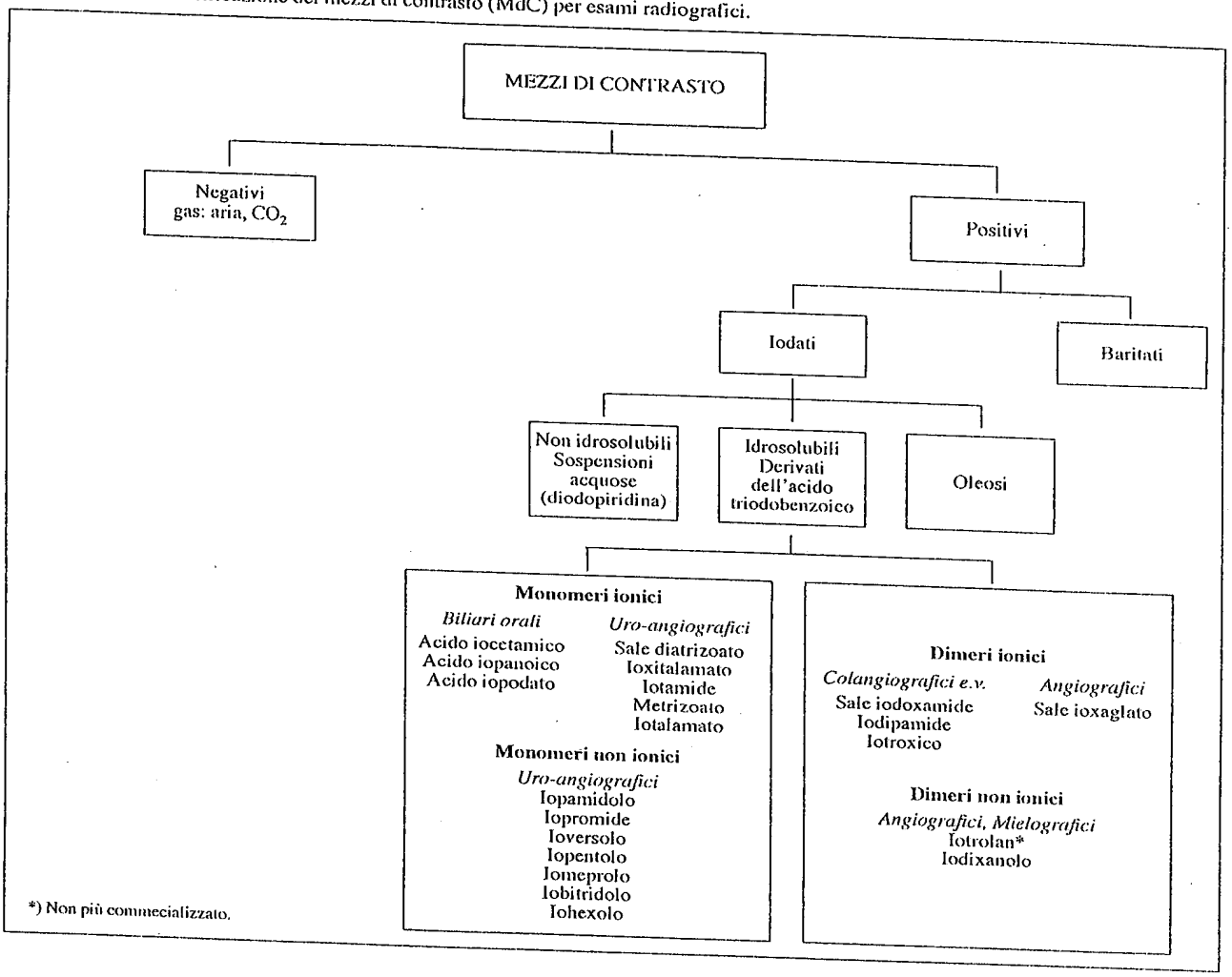
Fondamenti di chimica dei mezzi di contrasto radiografici

La scelta fondamentale della sostanza chimica utilizzata per veicolare atomi ad alto numero atomico è stata cruciale per lo sviluppo dei MdC. L'anello benzenico e lo iodio sono ancora la migliore combinazione per tutti gli obiettivi che devono essere garantiti per un MdC di larga utilizzazione, in quasi tutte le situazioni cliniche. L'anello benzenico assicura molti legami stabili chimici, sia con l'elemento pesante (I), sia con i radicali che influenzano fortemente le proprietà fisico-chimiche e farmacologiche del MdC (fig. 1). Esso lega in posizione 2-4-6 tre atomi di Iodio, mentre le posizioni 1-3-5 sono disponibili per le catene laterali, cui sono affidate le proprietà fisico-chimiche e biologiche. Lo iodio fra i vari elementi a più elevato peso atomico riunisce tre fondamentali proprietà:

— alto assorbimento Rx in rapporto alla «durezza» dei raggi utilizzati in diagnostica. L'energia di legame dell'or-

Dipartimento di Scienze Medico-Diagnostiche e Terapie Speciali - Università degli Studi di Padova - Sezione Radiologia - Azienda Ospedaliera-Università - Padova.

TABELLA I. — Classificazione dei mezzi di contrasto (MdC) per esami radiografici.



bita elettronica più interna è di 37 KeV e pertanto presenta il massimo assorbimento ai raggi X con 37 kVp, in più larga percentuale erogati da un carico fino a 80-90 kV al tubo radiogeno;

— elevato legame stabile con il benzene; veramente trascurabile è il numero di atomi di I che si possono liberare dalla molecola;

— bassa tossicità se combinato o eventualmente libero.

MdC convenzionali, ionici, molto ipertonici, ad elevata osmolalità

La molecola di base di numerosi prodotti (Angiografin®, Urografin® o Renografin®, Urovison®, Urovist®), l'acido diatrizoico, ha in posizione 1 il gruppo carbossilico -COOH salificato con Na^+ o con metilglucamina per ottenere una elevata solubilità. Un aumento di solubilità è assicurato anche dalle catene laterali in posizione 3 e 5. Esse consistono essenzialmente di idrogeno, ossigeno, carbonio e azoto con gruppi terminali carbossilici, amminici o più spesso ossidrilici (-OH) ai quali è affidata la proprietà di non legarsi alle proteine e di migliorare la tolleranza. La caratteristica fondamentale di tutti questi sali è costituita dalla solubilità per dissociazione elettrolitica, che produce due particelle ioniche (anione e catione) che richiamano molte molecole di acqua e innalzano l'osmolalità delle soluzioni (Osm/kg di acqua) rispetto a quella plasmatica. Perciò questa categoria di MdC tradizionali è definita ad alta osmolalità: high-osmolality contrast agent (HOCA). La loro utilizzazione ha rappresentato, dal punto di vista chimico, il grande vantaggio di ottenere concentrazioni anche molto elevate nelle soluzioni, fino a 80% e più, con viscosità relativamente bassa.

MdC a bassa osmolalità e non ionici

Nella decade degli anni '70 apparve chiaro che molti degli effetti collaterali dei MdC tradizionali erano da imputare più

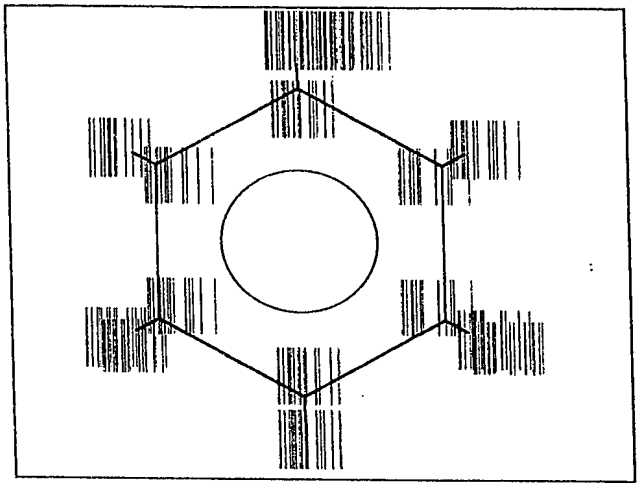


Fig. 1. — Anello benzenico: struttura di base dei MdC iodati (da Speck, mod.).

alla osmolalità elevata delle soluzioni, rispetto a quella plasmatica, che agli effetti chemiotossici del soluto. Pertanto la ricerca farmacologica profuse notevoli energie al fine di introdurre in radiologia diagnostica e interventistica agenti contrastografici che riducessero o annullassero l'incidenza di reazioni avverse. Un considerevole miglioramento fu infatti ottenuto con i MdC a bassa osmolalità, e particolarmente con quelli non-ionici: low osmolality contrast agent (LOCA) (tab. II).

Questi hanno dimostrato efficacia diagnostica equiparabile agli HOCA con pari concentrazione iodica, a fronte di una differente incidenza di reazioni avverse (AR). Anche l'unico agente ionico contrastografico a bassa osmolalità, lo ioxaglato, che è un dimero con produzione di due particelle ioniche in soluzione, ma con sei atomi di Iodio, è ritenuto simile ai LOCA non ionici (0.56 Osm/kg di acqua, rispetto a 0.6 Osm/Kg) anche se non sembra assicurare una bassa incidenza di reazioni avverse.

I vantaggi pratici dimostrati dai MdC non ionici rispetto a quelli ionici non sono solo limitati alla bassa osmolalità, ma sono riassumibili nei seguenti due aspetti:

1) l'incidenza delle reazioni generali, tipo nausea e vomito, nonché le reazioni simil-allergiche (allergic-like) dette anafilattoidi è più bassa, anche nelle manifestazioni più gravi rispetto ai MdC ionici; analoga osservazione non è possibile per le reazioni fatali, data la loro estrema rarità non valutabile statisticamente;

2) anche la neurotossicità è risultata minore rispetto ai MdC ionici, tanto da indurre molto precocemente una controindicazione all'uso di questi secondi MdC per somministrazioni nel sistema nervoso.

Tutti i vantaggi citati possono essere attribuiti alle seguenti proprietà chimiche:

— la molecola è priva di cariche elettriche;
— non contiene cationi di sodio o metilglucamina;
— la molecola è meglio schermata dalle catene laterali idrofiliche (fig. 2).

Tutte queste caratteristiche chimiche e particolarmente la presenza di catene laterali idrofiliche (fig. 2), determinano una ridotta tendenza della molecola a legarsi alle proteine, ad inibire gli enzimi e ad aderire alle membrane cellulari alterandone la funzione. Praticamente il paziente tollera meglio la somministrazione con riduzione di vomito, nausea, orticaria, edema delle mucose e minori effetti respiratori o cardiovascolari.

L'osmolalità ridotta, o quanto più possibile simile a quella del plasma, ha rappresentato il primo degli obiettivi da realizzare nello sviluppo di nuove formule di MdC. Poiché l'osmolalità è direttamente proporzionale al numero delle par-

TABELLA II. — *Mezzi di contrasto iodati.*

Classificazione	Nome commerciale	Casa farmaceutica
Monomeri ionici		
Diatrizoate	Gastrografin®,Selectografin®	Schering
Iothalamate	Angioconray®, Conray®	Bracco
Iodamide	Uromiro®	Bracco
Metrizoate		
Monomeri non ionici		
Iohexol	Omnipaque®	Nycomed-Amersham
Iopamidol	Iopamiro®	Bracco
Iobitridol	Xenetix®	Guerbet
Iopentol	Imagopaque®	Nycomed Amersham
Ioversol	Optiray®	Byk Gulden
Iomeprol	Iomeron®	Bracco
Iopromide	Ultravist®	Schering
Ioxilan	Oxilan®	Guerbet
Dimeri ionici		
Ioxaglate	Hexabrix®	Guerbet
Dimeri non ionici		
Iodixanol	Visipaque®	Nycomed Amersham
Iotrolan	Isovist®	Schering

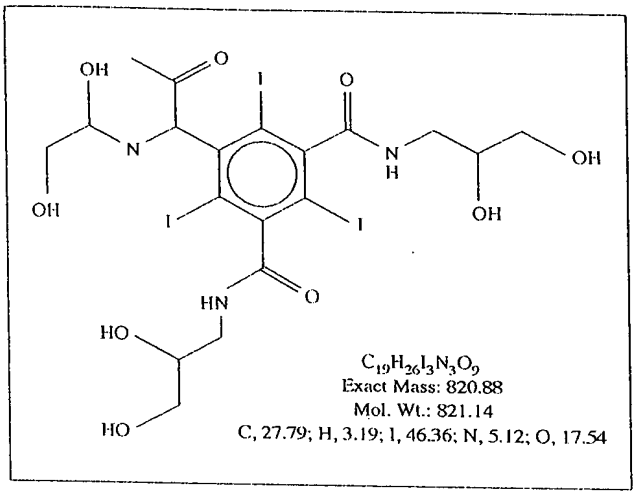


Fig. 2. — Struttura della molecola di monomero non ionico (iohexolo), con evidenziati i gruppi idrofili (-OH).

TABELLA III. — Classi dei MdC quale rapporto N. atomi di I/particelle in soluzione.

Composti	Atomi di iodio	N. particelle	Classe
Monomeri ionici	3	2	1,5
Monomeri non ionici	3	1	3,0
Dimeri ionici	6	2	3,0
Dimeri non ionici	6	1	6,0

ticelle in soluzione (ioni o molecole), essa può essere relativamente ridotta se aumentano gli atomi di iodio per particella in soluzione. Pertanto sono definite quattro classi di MdC relativamente a osmolalità/N. atomi di iodio secondo la successiva tabella (tab. III).

La quarta classe, l'ultima, è occupata da un solo MdC in commercio lo iodixanolo (Visipaque®) la cui osmolalità è pari a quella plasmatica, per cui questo MdC è effettivamente isotonico a 300mg I/ml. I vantaggi relativamente alle reazioni avverse sono stati di recente confermati (Aspelin P, 2003).

Catene laterali idrofiliche delle molecole dei MdC

Come sopra accennato la tossicità di una sostanza chimica è in gran parte causata dalla interazione con le proteine e le membrane cellulari. È accettato che le interazioni sono più facili con i gruppi o le componenti lipofiliche delle molecole. L'anello benzenico è fortemente lipofilo. I gruppi idrofilici sono rappresentati dagli ossidrili delle catene laterali e sembra che, quanto più numerosi essi siano, tanto più esaltino l'idrofilia della molecola e pertanto la sua tollerabilità. Inoltre è importante che i gruppi idrofilici siano bene distribuiti sulla superficie della molecola e che, nei vari isomeri della molecola, sia sempre mantenuta questa "protezione" da parte dei gruppi idrofilici o almeno, che essi si mantengano stabilmente bene distribuiti alla periferia dell'anello lipofilo (figg. 3, 4).

Le molecole infatti non sono piane ma grossolanamente sferiche nello spazio.

In quest'ultimo decennio è stata studiata in maniera approfondita la "struttura spaziale" delle molecole.

Il legame con le biomolecole dipende dalla carica elettrica presente nella molecola di MdC ionico; la molecola neutra del MdC non ionico ha minori affinità di legame. I legami più facili sono con i gruppi idrofili, come prima già affermato, ma possono essere favoriti anche dai ponti idrogeno che si realizzano ad esempio tra H e un altro atomo tendente a catturare elettroni, ad esempio l'ossigeno, come avviene nelle catene elicoidali degli acidi nucleici (Speck U, 1999). Un ponte idrogeno può realizzarsi tra molecola di MdC e peptide o catene peptidiche. In tali casi la molecola di MdC può legarsi a macromolecole tanto più quanto la sua concentrazione è elevata tale da favorire l'instaurarsi di ponti idrogeno (fig. 5)

Viscosità

La viscosità delle soluzioni di MdC è una misura della proprietà di fluire, fluidità, nei vasi e nei sistemi, aghi e cateteri di iniezione.

Essa si misura in millipascal × sec (la vecchia unità di misura era il centipoise), varia molto da un MdC all'altro, si innalza con l'incremento di concentrazione o con l'abbassamento della temperatura.

Una corretta valutazione deve essere riferita alla viscosità a 37°C: temperatura alla quale si deve portare il MdC prima dell'iniezione. Sono infatti disponibili contenitori per i flaconi di MdC alla temperatura di 37°C ove essi vengono mantenuti il giorno stesso del loro utilizzo. Tale precauzione previene l'eventuale formazione di cristalli nella soluzione, nel caso in cui essa si trovi a temperatura troppo bassa.

Un'alta viscosità deve essere tenuta in considerazione nell'iniezione selettiva di piccole arterie, poiché in tali casi si prolunga il contatto tra MdC ed endotelio, con possibili conseguenze, quali l'attacco della barriera emato-encefalica nelle iniezioni cerebrali, oppure un effetto vasodilatatorio in altri distretti microcircolatori.

I dimeri a parità di osmolalità possiedono una più elevata viscosità rispetto ai monomeri, essendo questa influenzata dal peso molecolare e dal numero dei gruppi idrossilici della molecola.

MdC colangiografici iniettabili

La formula di questi MdC è simile a quella degli uroangiografici per quanto riguarda il legame dello iodio all'anello benzenico; sono dimeri e ionici.

La iodipamide è il prototipo di questi contrasti: dimero diacido con un legame tra gli anelli benzenici e tra le due posizioni 3; questo legame non viene interrotto, cioè la molecola non viene metabolizzata e viene escreta con la bile senza subire modificazioni.

La posizione 5 dell'anello è libera e pertanto può legarsi per la sua lipofilia alle proteine plasmatiche, contrastando

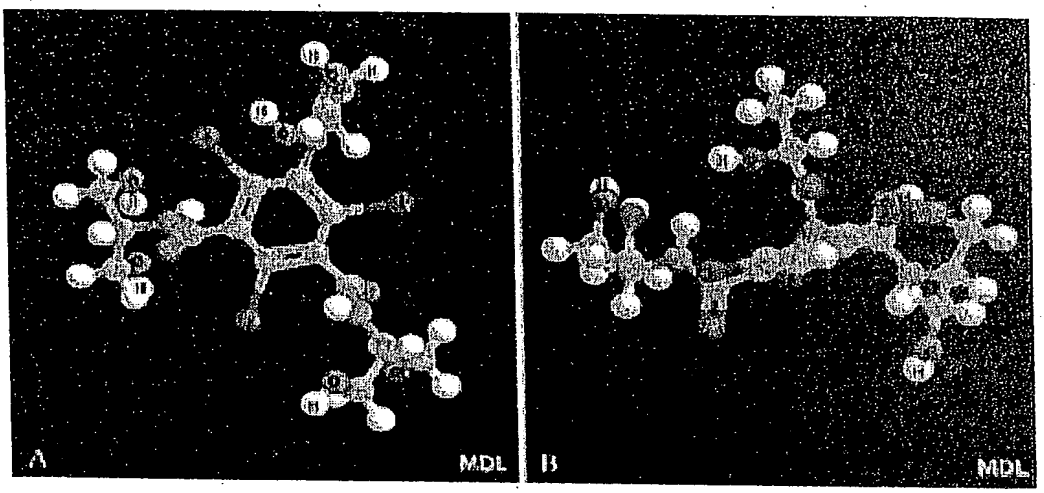


Fig. 3. — Rappresentazione tridimensionale (due visioni ortogonali) di molecola di monomero non ionico (Iopamidolo) con la distribuzione "in superficie" dei gruppi ossidrilici (-OH) e degli atomi di iodio (I) (da "3D molecular modeling", mod.).

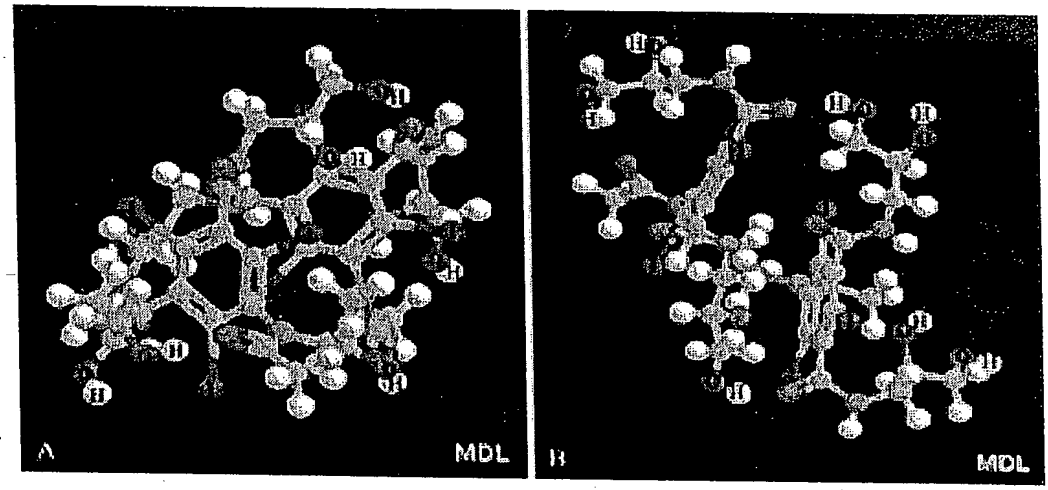


Fig. 4. — Rappresentazione tridimensionale (due visioni ortogonali) della molecola di dimero non ionico, con evidenziati i gruppi idrofilici (-OH) e gli atomi di iodio (I) (da "3D molecular modeling", mod.).

la filtrazione glomerulare, favorendo così la cattura da parte del fegato e la sua eliminazione come attivi metabolici naturali acidi.

Per queste caratteristiche chimiche un MdC colangiografico in un paziente portatore di paraproteinemia di Waldenström dimostrò una affinità così elevata con le proteine plasmatiche da generare una immediata "gelificazione" del sangue dal punto di iniezione, con decesso immmediato del paziente. Tale evento costituisce l'unico esempio di effetto letale osservato con questa patologia.

Le iniezioni dei MdC colangiografici, al fine di favorire il loro "legame" e trasporto con le proteine plasmatiche, vanno effettuate per infusione e.v. lenta, altrimenti la quota del MdC non legata subisce una escrezione urinaria ad ogni passaggio ematico attraverso il rene.

È ovvio che la possibilità di visualizzare le vie biliari è legata alla conservata funzionalità epatica, dalla quale dipende la capacità di eliminazione e pertanto anche la concentrazione del MdC nella bile (e la sua densità radiologica). La tolleranza del MdC deve pertanto essere elevata.

Quale indice di funzionalità epatica viene generalmente assunto il valore della bilirubinemia totale (indiretta e diretta), analogamente al valore della creatinemia o della sua clearance per i MdC iodati uroangiografici.

I MDC colangiografici iniettabili non sono più commercializzati in Italia.

Modalità di distribuzione del MdC iniettato

— Distribuzione (pool vascolare)
— Diffusione
— Eliminazione

Il MdC iniettato endovena si accumula nel plasma e la sua concentrazione plasmatica si innalza tanto più rapidamente quanto più veloce è l'iniezione.

Dal livello di concentrazione plasmatica dipende l'inten-

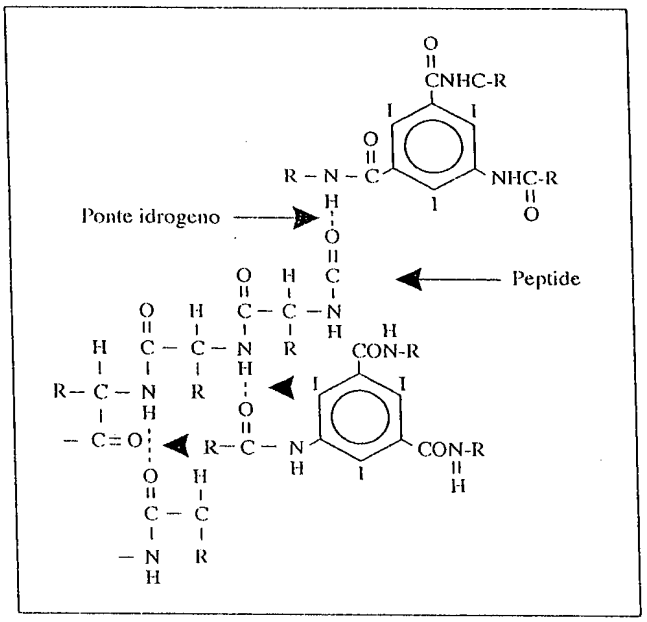


Fig. 5. — Esempio di possibile ponte idrogeno tra molecole di MdC e una catena polipeptidica (da Speck, mod.).

sità della ultrafiltrazione glomerulare al passaggio del plasma con il MdC attraverso il rene.

In una prima fase si osserveranno pertanto più contrastati i vasi, con la ovvia sequenza: dapprima le arterie e poi le vene di un organo o distretto.

In tale fase iniziale di distribuzione il MdC si evidenzia soprattutto, e può essere documentato solo con una TAC veloce, la componente ematica circolante, indicando in tal modo il "pool vascolare" che segnala anche il distretto parenchimale più vascolarizzato, separandolo da quello a minore perfusione, (come accade fra corticale renale e midollare) o la componente anatomica più vascolarizzata di una massa (angioma o neoplasia).

Contemporaneamente all'accumulo del MdC nel plasma l'elevata concentrazione plasmatica determina rapidamente il passaggio del MdC negli spazi extravascolari per la sua elevata diffusibilità e questo passaggio si continua fino a che sia raggiunta la stessa concentrazione tra sangue circolante e fluidi extravascolari e anche extracellulari, poiché il MdC non entra nelle cellule.

Questa quota costituisce il pool di diffusione che è molto più vasto di quello circolante. A tale fase corrisponde una distribuzione differenziata del MdC che si osserverà accumulato nei distretti a più elevata componente liquida extracellulare: ad esempio nei tessuti parenchimali anziché in quelli connettivali; ai primi corrisponde naturalmente anche una maggiore vascolarizzazione nella fase precedente.

Unica eccezione è il rene che presenta una parenchimografia a sempre maggiore intensità perché opera una clearance del plasma e trattiene il MdC ultrafiltrato nel distretto tubulare e canalare. Il rene infatti dimostra in assoluto il più alto "contrast enhancement" di tutti gli altri organi o tessuti.

L'eliminazione del MdC nell'urina permette una chiarificazione progressiva del sangue con riduzione della concentrazione plasmatica. La diffusione ora inverte la direzione e dai distretti extravascolari il MdC ritorna al plasma. Questa terza e ultima fase è molto prolungata, dura alcune ore fino a che tutto il MdC è stato ultrafiltrato dal rene.

Sterilità e conservazione del MdC

La sterilità dei MdC è ottenuta con processi di filtrazione e ultrafiltrazione che eliminano le molecole di peso molecolare superiore a 10.000 D. Ciò garantisce che non solo i microrganismi, ma anche i loro prodotti metabolici e le sostanze piogene vengano eliminati.

Nella preparazione delle infusioni e dei dispositivi di iniezione va mantenuta la sterilità.

Solo i MdC ionici hanno una azione antimicrobica dovuta alla alta osmolalità ed alla più cospicua chemiotossicità. I MdC non ionici sono invece dei buoni terreni di coltura e pertanto una confezione aperta non deve essere conservata più della sessione di lavoro o oltre il giorno stesso della sua apertura.

I trasferimenti del liquido da ampolle in siringhe, contenitori e iniettori devono rigorosamente impedire il contatto con superfici non sterili, quali ad esempio il bordo del contenitore originale: è necessario pertanto rimuovere il tappo e prelevare il MdC con una cannula sterile, richiudere il contenitore per i successivi prelievi, se la confezione è di discreta quantità, evitando di reintrodurre il liquido aspirato nel contenitore originale. Anche per confezioni di grande volume (500 ml), utilizzate per esempio nelle sale angiografiche, si devono usare appositi sistemi di prelievo chiusi per caricare gli iniettori, anche al fine di evitare contaminazioni con materiali e ingredienti corpuscolati, ad esempio di plastica liberatisi da manipolazioni plurime. Può essere anche raccomandato l' impiego di siringhe già riempite di MdC per evitare la fase di aspirazione dalle fiale o ampolle.

Il MdC non va ovviamente riutilizzato anche per una certa instabilità chimica del prodotto, soprattutto alle elevate temperature.

La conservazione delle confezioni sterili di MdC deve rispondere a due condizioni importanti:

a) le soluzioni contenute in ampolle trasparenti vanno immagazzinate, protette dalla luce per evitare l' azione di degrado indotta dalle radiazioni UV. Pertanto le ampolle vanno rimosse dalle loro scatole solo al momento dell' utilizzo e conservate in luoghi bui.

b) meno rigida è la raccomandazione di tenere le confezioni di MdC al riparo dalle radiazioni X: il rifornimento nella sala radiologica non deve tuttavia determinare un lungo periodo di conservazione dei MdC all' esposizione radiante.

Infine prima dell'uso la confezione in vetro trasparente va esaminata a luce trasmessa per evidenziare eventuali impurità, molecole estranee o cristalli di sale precipitato.

TABELLA IV. — Caratteristiche delle reazioni avverse chemiotossiche e anafilattoidi.

	Reazioni avverse	
	Chemiotossiche	Anafilattoidi
Prevedibili	Sì	No
Dose-dipendenti	Sì	No
Fattori di rischio	Cardiopatie, nefropatie, epatopatie, encefalopatie	Atopia, precedenti reazioni a MdC
Pretrattamento con steroidi	Non efficace	Utilità discussa

Eventi avversi

In seguito alla somministrazione parenterale di un MdC iodato uroangiografico si possono verificare eventi avversi, indesiderati o inattesi.

Essi si configurano come eventi nuovi, occorsi durante o dopo l'intervento diagnostico o interventistico, caratterizzati dal produrre un cambiamento attuale o potenziale dello stato di salute del paziente. Nell'ambito degli eventi avversi vanno distinte le reazioni avverse (adverse reactions, AR) attribuibili alla somministrazione dell'agente contrastografico, dalle complicanze imputabili all'invasività della procedura o al preesistente stato clinico del paziente (ad esempio la perforazione vasale in seguito a cateterizzazione o la reazione vaso-vagale).

Incidenza

Sono stati condotti studi atti a stabilire la frequenza di eventi avversi. Fondamentale è quello di Katayama (1990) eseguito su un numeroso campione (337.647 pazienti) suddiviso in due gruppi di pazienti sottoposti ad indagine radiologica contrastografica con MdC rispettivamente ionico e non ionico.

Lo studio mirava a verificare i vantaggi del MdC non ionico e l'importanza del fattore osmolalità nel ridurre l'incidenza delle reazioni avverse. Le reazioni avverse da MdC ionico risultavano quattro volte più frequenti di quelle da MdC non ionico, con un'incidenza rispettivamente del 12,66% contro il 3,13%. Ancora più marcata la differenza per le reazioni gravi o molto severe: l'incidenza era rispettivamente del 0,22% contro lo 0,04%.

Seguirono polemiche da parte nordamericana (Bettmann MA, 1990, 1996, 1997) ai risultati dello studio di Katayama. Le sue conclusioni erano state peraltro anticipate da un altro autore dell'area del Pacifico orientale, l'australiano Palmer FJ (1988), e successivamente riconfermate da altri, l'ultimo fu Cochran (2002). Le osservazioni di Katayama sono divenute pertanto il termine comune di riferimento per la progressiva diffusione e la definitiva affermazione dei MdC non ionici.

Classificazione

Le reazioni avverse (Tab. IV) si suddividono in:

— Chemiotossiche (tipo A). Tali reazioni sono dipendenti dalla dose e dalla concentrazione plasmatica del farmaco, perciò potenzialmente prevedibili. Sono influenzate dalle caratteristiche del MdC, come l'osmolalità, la viscosità, l'idrofilia.

— Anafilattoidi (tipo B, allergic-like) non dose-dipendenti, imprevedibili, che possono indurre il rilascio di istamina o altri mediatori biologici come serotonina, prostaglandine, bradichinina, leucotrieni, adenosina e endotelina, solitamente attivi nei fenomeni allergici.

A seconda della loro severità le reazioni avverse vengono suddivise in:

— lievi (con frequenza del 5%): sapore metallico in bocca, sensazione di calore, nausea e vomito, sudorazione, disestesia periorale, sensazione di testa leggera, dolore nella sede dell'iniezione, orticaria, emicrania;

— moderate (con frequenza dello 0,022%): persistenza ed aumento di intensità dei sintomi minori, dispnea, ipotensione, dolore toracico;

— severe (con incidenza dello 0,0025%): tosse, starnuti, broncospasmo, ansia (sintomi minori). Inoltre: diarrea, parestesie, edema al volto, alle mani ed in altri siti corporei, dispnea, cianosi, edema della glottide, ipotensione marcata, bradicardia, shock, edema polmonare, aritmie, midriasi, convulsioni, paralisi, coma, morte.

Generalmente le reazioni compaiono entro un'ora dall'iniezione del MdC e sono definite immediate; se si verificano dopo un'ora dall'iniezione fino al massimo di sette giorni dalla somministrazione sono definite ritardate. Queste seconde sono meno frequenti delle prime, e verranno trattate successivamente.

Meccanismi fisiopatologici delle reazioni chemiotossiche

Le reazioni chemiotossiche sono legate alla tossicità intrinseca della molecola e dipendono fondamentalmente dalle sue caratteristiche chimico-fisiche e dalla dose somministrata.

L'alta osmolalità è uno dei principali fattori implicato nella patogenesi delle reazioni da MdC iodato (Katayama H, 1990).

Ogni sostanza iniettata, con osmolalità significativamente diversa da quella plasmatica ha un effetto sulle sostanze con le quali viene in contatto, quali l'endotelio, le cellule ematiche, altre membrane cellulari e macromolecole (es. proteine).

Si ritiene che talvolta la tossicità sia, almeno in parte, dovuta all'interazione degli agenti contrastografici con macromolecole circolanti o con cellule. Le porzioni idrofobiche delle molecole del contrasto, principalmente l'anello ben-

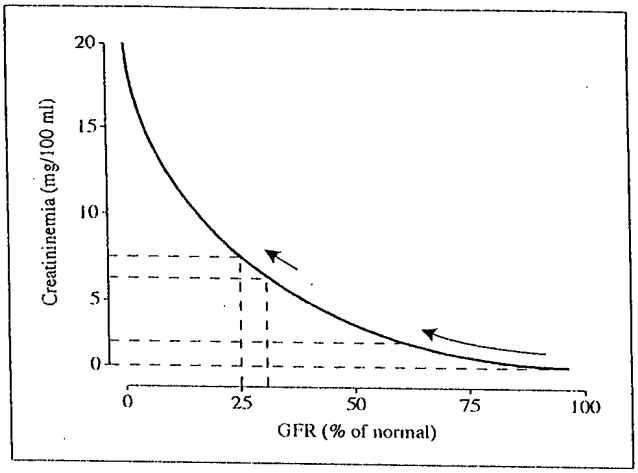


Fig. 6. — Relazione tra concentrazione sierica della creatinina (mg/dl) e filtrato glomerulare (GFR).

zenico, possono interagire mediante una combinazione di forze di Coulomb e interazioni idrofobiche non-specifiche, con porzioni lipofiliche delle membrane cellulari e di macromolecole biologiche. Tale capacità di interazione dipende anche dalla forma tridimensionale della molecola: proteggendo cioè i gruppi lipofilici (anello benzoico) con gruppi idrofilici, può essere ridotta la probabilità di interazione con le membrane cellulari e con le macromolecole, in termini estensivi decresce la probabilità di evento avverso (figg. 3, 4).

La bassa tossicità clinica degli agenti contrastografici non-ionici è ascrivibile alla loro ridotta osmolalità e bassa chemiotossicità, nonché all'assenza di ioni sodio.

Gli organi principalmente colpiti nelle reazioni chemiotossiche sono: il rene, il sistema nervoso centrale e l'apparato cardiovascolare.

Rene

Il rene rappresenta il bersaglio privilegiato dei tossici poiché essi sono eliminati completamente dall'emuntorio renale tramite la filtrazione glomerulare, pertanto la concentrazione intratubulare del farmaco può essere molto più alta della concentrazione plasmatica. Si pensi infatti come alla TC il rene sia l'organo a più elevato contrast enhancement. Inoltre il meccanismo tubulare di trasporto degli ioni può favorire l'entrata dei tossici all'interno delle cellule tubulari.

I principali meccanismi potenzialmente responsabili della nefrotossicità sono quello vascolare e la tossicità diretta, sia a livello glomerulare che tubulare.

In seguito alla somministrazione di MdC il flusso renale presenta dapprima un transitorio aumento, apparentemente correlato all'iperosmolalità, come nel circolo periferico. Ad esso segue una prolungata vasocostrizione con conseguente diminuzione della perfusione renale e del GFR (glomerular filtrate rate), con accentuazione delle condizioni ipossiche a livello della midollare.

I più importanti fattori implicati in questa fase sono:

— Il feedback tubuloglomerulare: il MdC iperosmolare ultrafiltrato incrementa la diuresi e la natriuresi.

— La presenza del MdC iperosmolare all'interno di tutto il sistema tubulare renale trattiene acqua.

— Ruolo fondamentale potrebbe avere l'endotelina rilasciata dalle cellule endoteliali danneggiate, che determina vasocostrizione.

Per quanto riguarda l'effetto nefrotossico, esso si estrinseca sia con un'aumentata permeabilità della membrana basale glomerulare e conseguente proteinuria sia con danno tubulare. Le molecole di MdC, infatti, non essendo riassorbite ristagnano all'interno del tubulo in concentrazione tanto maggiore quanto più intensa è la disidratazione del soggetto e quindi quanto più è marcato il riassorbimento tubulare dell'acqua. Tale effetto è evidenziato da una temporanea e aumentata enzimuria e dalla vacuolizzazione delle cellule del tubulo prossimale, alterazioni che rappresentano una normale risposta al MdC e non hanno rilevanza clinica (Berg KJ, 1993).

Nelle urine dei pazienti sottoposti ad esame contrastografico infatti si osservano cellule epiteliali tubulari, sedimenti amorfi, cristalli a stampo derivati dai frammenti cellulari ed una proteinuria di grado moderato.

Caratteristiche cliniche della nefrotossicità

Da un punto di vista clinico nella maggior parte dei casi la nefrotossicità è asintomatica; soltanto una sistematica sorveglianza di laboratorio dopo la procedura contrastografica può consentire di individuare le alterazioni della funzione renale.

Si verifica un incremento della concentrazione della creatinina sierica del 25% in termini relativi o di 0,44 umol/L (0,5 mg/dl) in termini assoluti. Tuttavia la creatinina sierica non è certo il parametro ideale di funzionalità renale, in particolare del filtrato glomerulare, poichè dipende dal sesso, dall'età e dalla massa muscolare. Può inoltre non essere elevata fino ad una riduzione del GFR del 50% per l'incremento compensatorio della sua secrezione tubulare (Becker J, 1991).

È certo che modeste alterazioni della sua concentrazione non possiedono valore predittivo rispetto alla possibile nefrotossicità da MdC. Tuttavia la creatinina rappresenta un indice più che sufficiente di funzionalità renale e, comunque, preferibile all'azotemia.

Combinando i valori di creatininemia e di VFG l'adattamento della funzione renale, pur essendo minimale o nullo nei confronti dell'acccumulo di urea o creatinina nel sangue, richiede gradi molto marcati di riduzione della VFG perché la creatininemia o l'uremia diano segnale molto netto di insufficienza renale (fig.6).

La valutazione della clearance della creatinina viene calcolata semplicemente mediante la formula di Cockcroft-Gault (Cockcroft DW, 1976):

$$\text{Cl Cr (ml/min)} = \frac{(140\text{-età}) \times \text{peso(kg)}}{72 \times \text{Cr pl (mg/dl)}}$$

e consente una valutazione del GFR indipendente dai fattori costituzionali (×0,85 nelle donne).

L'incremento del livello della creatinina può raggiungere il picco nei 4/5 giorni che seguono la procedura e rientrare nel

range di normalità entro una o due settimane con ripristino completo della funzionalità renale (Morcos SK, 1998, 2001).

Oltre agli esami di laboratorio può essere utile il rilievo radiologico della cinetica di eliminazione renale del MdC: la persistenza dell'effetto nefrografico depone per nefrotossicità se i valori densitometrici persistono superiori a 113 U.H. dopo 24 ore dalla somministrazione del MdC (Love L, 1994)

Il ricorso alla dialisi comunque è eccezionale e richiesto solo nei pazienti ad alto rischio, cioè con IRC o con particolare vulnerabilità renale.

La nefrotossicità dei MdC è stata, soprattutto nel passato, enfatizzata. Le alterazioni qui sopra descritte, meno accentuate per i LOCA, determinano solo raramente (meno dell'1%) disfunzione renale in soggetti senza precedenti disfunzioni d'organo.

I principali fattori di rischio per la nefrotossicità possono essere distinti in fattori:

1) correlati al paziente:

— insufficienza renale cronica (IRC);

— disidratazione;

— diabete mellito associato a IRC;

— ipovolemia secondaria a scompenso cardiaco o sindrome nefrosica;

— farmaci nefrotossici (FANS; aminoglucosidi; cisplatino);

2) correlati alla procedura: ripetute indagini in breve tempo:

— alte dosi di MdC.

La combinazione di diabete mellito e insufficienza renale aumenta il rischio di nefropatia da MdC di almeno il doppio rispetto al caso in cui vi sia solo l'insufficenza renale (Becker J, 1991; Solomon R, 1998).

È noto che l'insufficenza renale è più grave in condizioni di disidratazione. Il rene infatti nell'IRC mantiene un discreto equilibrio solo mantenendo una elevata diuresi, cioè sostenendo una elevata poliuria. Il limite del rene in quelle situazioni è soprattutto l'aver perduto parte della capacità tubulare di concentrare l'urina.

Pertanto la funzionalità renale se misurata in fase di disidratazione risulta più grave che non nella fase ordinaria di elevata idratazione.

La stessa precauzione va considerata nel caso di ipovolemia cardiogena o di sindrome nefrosica.

È evidente come una latente o modesta insufficienza renale diventi più grave nelle condizioni di carenza di liquidi. A tale proposito vale la pena richiamare una caratteristica cruciale della funzione renale articolata su due tipi di nefroni. Il nefrone della corteccia più esterna (outer cortex) è il nefrone con ansa di Henle più corta e ha la caratteristica funzionale di essere sodio e idro-disperdente.

Il nefrone con glomerulo situato nella corticale più vicina alla midollare (inner cortex) è quello con ansa di Henle lunga e con impronta funzionale di idroritenzione (fig. 7).

L'attivazione maggiore di un tipo o dell'altro nefrone dipende anche dall'osmolalità del sangue. Infatti nelle condizioni di shock emorragico così come in quelle nelle quali sono attivati i sistemi adrenergici si dimostrano una vasocostrizione e una ridotta perfusione dei nefroni dell'outer cortex. Analogo fenomeno si osserva sotto carico di sostanze steroidee. L'idratazione del plasma richiama invece alla funzione questi nefroni esclusi. È evidente che con questa attivazione è più agevole l'escrezione dei soluti ipertonici e in particolare dei MdC.

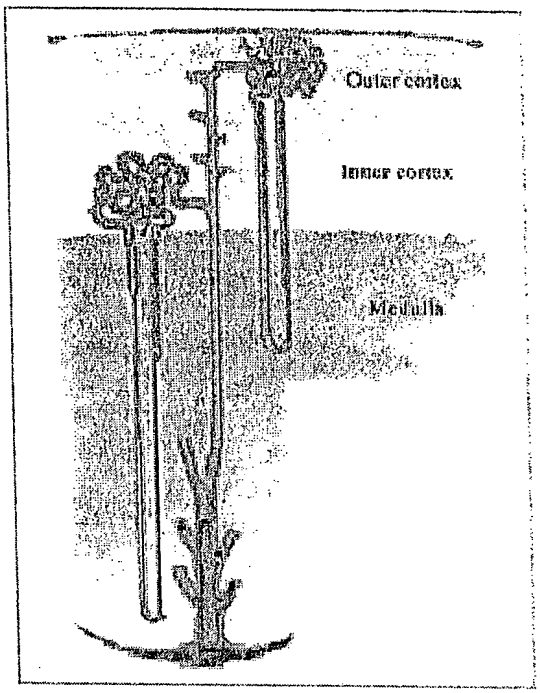


Fig. 7. — Struttura dei due tipi di nefroni corticali.

Si conclude dicendo che i diuretici osmotici, nonché quelli vasoattivi come la furosemide, e i calcioantagonisti attivano proprio l'outer cortex. Gli altri composti come quelli steroidei, nonché le sostanze adrenergiche permettono invece solo la funzione dell'inner cortex con ritenzione di acqua e di soluti.

Allo scopo di ridurre l'incidenza dell' insufficenza renale nei pazienti a rischio è opportuno pertanto assumere determinate precauzioni:

1) identificare e correggere eventuali fattori di comorbidità;

2) nei pazienti ad alto rischio usare MdC a bassa osmolalità;

3) assicurare eventualmente una adeguata idratazione mediante l'infusione di soluzione salina 0,45% 1 ml/kg/h da continuare per 24 ore dopo l'esecuzione dell'esame (attenzione nei pazienti con insufficienza ventricolare sinistra per evitare un edema polmonare);

4) abolire la disidratazione prima della procedura;

5) evitare, se possibile, il contemporaneo utilizzo di farmaci nefrotossici (steroidi, FANS, aminoglicosidi);

6) nei pazienti con fattori di rischio calcolare un sufficiente intervallo di tempo prima di ripetere una successiva iniezione e comunque ridurre le dosi per quanto possibile;

7) ricordare che l'incremento della concentrazione della creatinina è ritardato (circa 4 giorni);

8) nei pazienti dializzati programmare la seduta di emodialisi al termine dell'esame diagnostico.

Sistema nervoso centrale

La neurotossicità riflette direttamente le caratteristiche intrinseche della molecola ed è indicata come uno dei fattori responsabili delle reazioni avverse indotti dai MdC.

Alcuni studi sperimentali su modelli animali hanno suggerito la relazione del danno cerebrale con l'osmolalità e con la chemiotossicità della molecola somministrata. Peraltro queste alterazioni avvengono a volumi molto più elevati di quelli usuali in campo clinico. L'ipertonicità del MdC si è chiaramente rivelata tossica per il tessuto cerebrale, dipendendo gli effetti dalla sede di alterazione della barriera.

La possibilità di passaggio del MdC all'interno dei distretti cerebrali dopo somministrazione intravascolare non è impossibile, considerato che la barriera emato-encefalica non è una struttura anatomica fissa ed immutabile. La permeabilità di questa barriera può essere modificata da trattamenti farmacologici, da aumenti dell'osmolarità o della viscosità plasmatiche dopo iniezione di MdC o a causa di condizioni patologiche come vasculopatie cerebrali, metastasi cerebrali, traumi, alcolismo o tossicodipendenza. È essenziale l'importanza dei contatti o dei legami della molecola del MdC con le membrane cellulari. La lipofilicità dell'anello benzenico o dei gruppi carbossilici può indurre il superamento o il danno della BEE anche se introdotti nel sangue, mentre sarà più facile tale danno se introdotti direttamente a contatto con le strutture nervose come nella mielografia.

A ciò si aggiungeva il danno potenziale dei ioni-sodio nelle salificazioni dei gruppi carbossilici (-COO$^-$ Na$^+$) dei MdC ionici.

L'eliminazione di questi inconvenienti è attuata utilizzando la salificazione con glucamina anziché con sodio (-COO-glucam.) nei MdC ionici, o sostituendo i gruppi carbossilici con gruppi idrossilici (-OH) e incorniciando il nucleo molecolare benzenico del MdC con catene a terminali idrossilici per ottenere una più completa idrofilia della molecola stessa.

Apparato cardiovascolare

Gli effetti dei MdC sul sistema cardiovascolare sono diminuiti ma non eradicati con l'uso dei LOCA. Essi possono inoltre variare a seconda del sito di iniezione.

Cuore. — L'iniezione del MdC intraventricolare aumenta la frequenza cardiaca; al contrario lo studio coronarico selettivo induce bradicardia. L'immissione del MdC nelle arterie coronarie può inoltre precipitare una varietà di aritmie, quali la fibrillazione ventricolare, la tachicardia ventricolare o anche l'asistolia in particolare quando avviene nell'arteria di destra (Levin DC, 1992). Più comuni sono le variazioni dell'ECG, in particolare l'allungamento della conduzione atrioventricolare.

Le reazioni avverse si manifestano in modo più severo in pazienti con recente infarto miocardio, coronaropatia, angina instabile, operati di by-pass o con scompenso ventricolare sinistro.

Il principale mediatore di tali effetti, oltre all'osmolalità e alla chemiotossicità, è il contenuto di ioni (Na$^+$, Ca^{++}) della soluzione iodata, responsabile anche dell'effetto inotropo negativo dei MdC.

L'edema polmonare è dovuto al sovraccarico del circolo indotto dall'iperosmolalità. In rari casi tuttavia ne è stata dimostrata la natura allergica (vedi oltre).

Circolo periferico. — La pressione arteriosa diminuisce sia per l'effetto vasodilatatore del MdC sia per l'induzione di un riflesso colinergico che induce anche bradicardia; l'atropina rappresenta infatti il farmaco di prima scelta nelle crisi ipotensive da MdC.

Sul versante venoso da segnalare il rischio di tromboflebite in seguito allo studio selettivo del circolo venoso, dovute al danno endoteliale diretto del MdC.

Meccanismi fisiopatologici delle reazioni anafilattoidi

L'altro grande gruppo di reazioni avverse, sono le reazioni anafilattoidi non dipendenti dalla dose (Dewachter P, 2001).

Non esiste una definizione universalmente accettata di reazione anafilattica e anafilattoide (Tab. V).

Molti meccanismi possono determinare gravi sintomi o segni scatenati dalla attivazione di mastcellule e basofili. Il termine anafilassi è generalmente utilizzato per le reazioni da ipersensibilità mediate dalle immunoglobuline E.

Le reazioni definite anafilattoidi sono simili ma non dipendono dall'ipersensibilità.

Anche queste come quelle anafilattiche, sono di varia gravità (Tab. VI), rapidità e progressione; raramente sono bifasiche o persistono per più di 24 ore.

È molto comune, per semplicità, che il termine anafilattico sia utilizzato per i due tipi di reazione, anche perché la comparsa clinica e il trattamento sono simili. La distinzione deve essere fatta per il follow-up immediato.

La clinica di alcune reazioni avverse al MdC ha spesso indotto a credere all'ipotesi immunomediata poiché i quadri clinici mimavano esattamente le risposte allergiche (orticaria, asma, edema della glottide, ecc.). Le reazioni anafilattoidi si verificano un pò più frequentemente in pazienti che hanno avuto precedenti reazioni e in pazienti severamente allergici ad antigeni ambientali e tali pazienti presentano meno frequentemente le reazioni se sottoposti ad indagini con l'utilizzo di mezzi di contrasto non ionici.

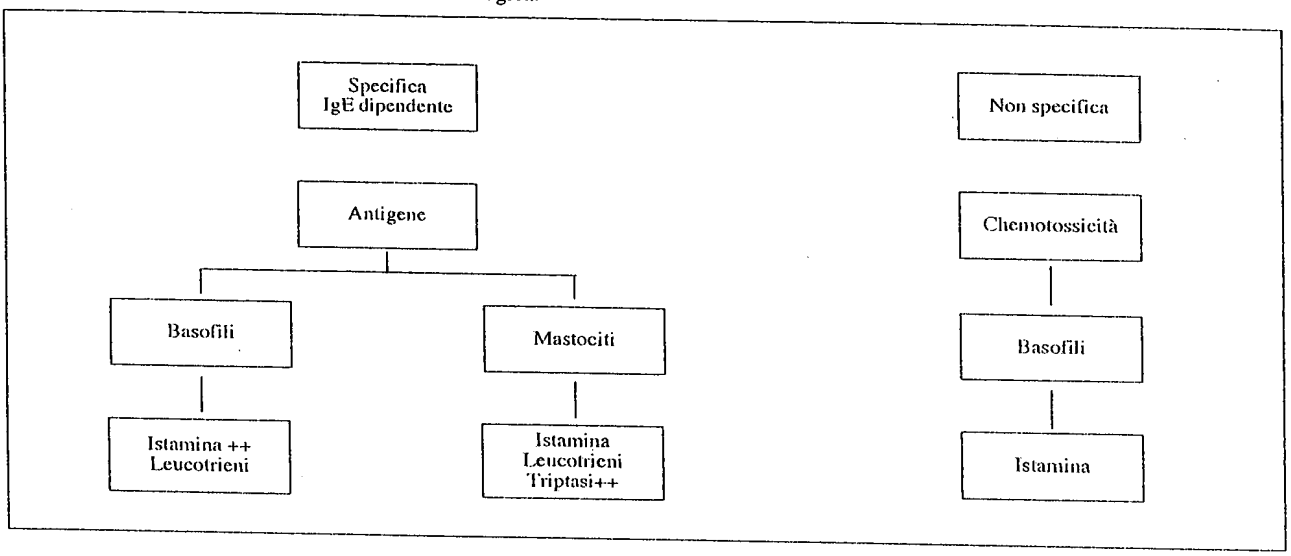
A partire dagli anni '70 numerosi studi hanno cercato di dimostrare i meccanismi fisiopatologici di questo tipo di reazioni avverse.

In quegli anni sono stati compiuti studi *in vivo* dopo l'iniezione di mezzi di contrasto iodati che hanno evidenziato un incremento del livello di istamina e dei fattori del complemento sia nei pazienti che avevano avuto una precedente reazione ai MdC sia nei pazienti che non avevano mai avuto tali reazioni, senza differenze significative tra i due gruppi.

Uno degli ultimi è lo studio di Laroche (1998) che ha ipotizzato l'intervento di meccanismi cellulo-mediati o reazioni di ipersensibilità di tipo III con coinvolgimento di immunocomplessi.

Inoltre, analogamente alle reazioni allergiche, i sintomi clinici di tali reazioni sono sostenuti dalla liberazione dei mediatori dell'anafilassi quali l'istamina e gli SRS-A (Slow-

TABELLA V. — Meccanismi di attivazioni immunologica.



Reacting Substance of anaphylaxis). La prima viene rapidamente rilasciata in circolo in seguito all'attivazione dei mastociti ed ha breve emivita. Gli SRS-A, tra cui le triptasi, hanno una emivita maggiore nel circolo ematico e perciò dosabili con maggiore attendibilità.

Entrambe queste sostanze sono state riscontrate nel plasma in concentrazione aumentata nei soggetti reattivi e quella dell'istamina è risultata correlata alla gravità dei sintomi.

Tali sostanze sono liberate dalle mastcellule e dai basofili che possono essese attivati, oltre che per meccanismo Ig-E mediato, anche da altre sostanze, tra le quali i fattori del complemento. Quest'ultimi sebbene siano stati riscontrati in concentrazione elevata in soggetti con reazioni avverse ai MdC, sembra siano una delle conseguenze della liberazione delle triptasi e sarebbero implicati nell'attivazione dei mastociti.

Solo recentemente Laroche *et al* (1999) hanno dimostrato come in alcuni casi di reazioni ai MdC fossero presenti anticorpi della classe IgE. L'interazione delle Ig-E con le molecole di MdC sarebbe mediata dalle proteine, interazione più probabile con i MdC ionici.

Tuttavia studi più recenti dello stesso autore hanno evidenziato come solo una piccola percentuale delle reazioni avverse severe (circa il 3%) siano reazioni anafilattiche vere, cioè caratterizzate dalla presenza di IgE specifiche per le molecole di MdC nel circolo ematico.

Nello stesso lavoro Laroche giunge alla conclusione che dobbiamo distinguere due tipi di reazioni "allergic-like": quelle lievi, di gran lunga più frequenti, caratterizzate dalla liberazione dei mediatori dell'anafilassi a livelli non elevati, in soggetti sensibili; tale liberazione avverrebbe attraverso un meccanismo non Ig-E mediato, ma per tossicità diretta della molecola di MdC sui mastociti.

La dizione di "anafilattoide" rimane quindi ancor oggi la più appropriata per queste reazioni.

TABELLA VI. — Gradi delle reazioni anafilattiche e anafilattoidi.

Grado I	Solo segni cutanei: orticaria, eritema diffuso
Grado II	Come I più nausea, tosse, dispnea, tachicardia, ipotensione
Grado III	Come II più vomito (non solo conati), diarrea, broncospasmo, cianosi, shock
Grado IV	Arresto cardiaco

La liberazione di istamina e degli altri mediatori attraverso un meccanismo Ig-E mediato avverrebbe nelle rare reazioni allergiche propriamente dette. Questo tipo di reazioni sono di gravità severa, ad alto rischio di ricorrenza e non prevenibili con i corticosteroidi.

Un successivo studio su pazienti sottoposti ad indagine con un dimero ionico ha dimostrato che solo il 2,4% dei pazienti, con una reazione severa, aveva una positiva IgE-RIA. Questo studio retrospettivo ha confermato che l'anafilassi IgE mediata è rara, ma è uno dei possibili meccanismi delle reazioni avverse, come dimostrato anche da Mita *et al* (1998). Gli organi in cui si verificano eventi avversi con caratteri clinici analoghi alle razioni anafilattiche sono la cute, l'apparato respiratorio e quello gastroenterico.

Reazioni muco-cutanee

Sono nella maggior parte dei casi di tipo anafilattoide: prurito, flushing, eritema, angioedema e orticaria, quest'ultima caratterizzata da ponfi pruriginosi con area centrale pallida, solitamente localizzate al volto, al collo ed al torace. Se circoscritta è una reazione autolimitantesi che si risolve spontaneamente o con una singola dose di antistaminici.

La congestione delle mucose può determinare lacrima-

TABELLA VII. — Storia positiva di asma ed eventi avversi in 32294 procedure vascolari (Campus Link, 1994).

		Eventi avversi in asmatici		
		AE (%)	Reazioni avverse (%)	Complicanze (%)
Asma	No	2,5	1,0	1,4
	Sì	2,8	1,0	1,3

zione, rinorrea ma anche edema della glottide che può richiedere l'immediata intubazione.

Sono però considerate chemiotossiche manifestazioni tipo il senso di calore e il dolore conseguente all'iniezione, dovuti alla carica elettrica ma soprattutto all'osmolalità del MdC. Molti MdC non ionici alla concentrazione di 300 mg I/ml hanno valori di osmolalità prossimi al valore indicato come soglia del dolore (600 mOsm/kg).

Inoltre l'extravasazione del MdC, pur non rientrando nelle reazioni avverse, è una delle complicanze più frequenti che può portare a celluliti chimiche, trombosi venosa, eruzione bollosa fino a sindromi compartimentali. Il trattamento conservativo prevede il sollevamento dell'arto, impacchi caldi, e nei casi più severi l'iniezione di jaluronidasi, la somministrazione di corticosteroidi e antibiotici o il ricorso alla chirurgia.

Apparato respiratorio

La somministrazione di mezzo di contrasto provoca lieve e subclinica broncocostrizione delle vie aeree inferiori. L'asma è stata generalmente considerata una delle manifestazioni allergic-like poiché è stata valutata un'incidenza nel 1,88% e 0,23% dei casi rispettivamente nelle esposizioni ai MdC ionici e non ionici (Katayama H, 1990). Molti altri autori hanno prima e dopo la segnalazione di Katayama sempre riportato l'asma come un fattore di rischio quantificabile nelle esposizione ai MdC (Morcos, 2001). Altri autori e anche chi scrive hanno per contro confutato questa affermazione, segnalando come in un trial nazionale (Campus Link 1994) non è stata osservata una maggiore incidenza di reazioni avverse in pazienti con storia di asma bronchiale (Mikkonen, 1995; Feltrin 1997) (Tab.VII).

È peraltro indubbio che l'assenza di criteri oggettivi per segnalare l'esistenza di vero asma bronchiale, sia nelle pubblicazioni a favore che in quelle contrarie, rende tale condizione di incerta classificazione.

Ulteriore complicanza alla somministrazione di MdC è rappresentata dall'edema polmonare da anafilassi. Si tratta di un edema di membrana associato a deplezione di volume. Pur essendo molto meno frequente, è essenziale distinguerlo da quello cardiaco poiché i farmaci diuretici sono in questo caso controindicati (Mare K., 1984).

Apparato gastroenterico

I sintomi più frequenti a carico del tratto gastroenterico sono nausea, vomito, dolore addominale crampiforme e diarrea, dovuti alla bassa idrofilia del MdC o espressione di reazione anafilattica.

Reazioni ritardate

Un cenno a parte meritano le reazioni ritardate che insorgono in un intervallo di tempo variabile da 1 ora dopo l'iniezione del MdC ad alcuni giorni successivi.

In letteratura è riportata una frequenza che varia dallo 0,5 al 2% e nella maggior parte dei casi si tratta di reazioni minori, transitorie e autolimitantesi.

Consistono più frequentemente in eruzioni cutanee (esantema maculo-papulare, orticaria, angioedema), sindrome simil-influenzale, disturbi gastrointestinali, dolori agli arti. Tali reazioni sono risultate più frequenti nei pazienti in trattamento con interleukina 2; ciò è uno degli elementi a supporto di una reazione di ipersensibilità ritardata di tipo cellulo-mediato nella genesi di queste reazioni (Christiansen C, 2000, 2002). È stato inoltre osservato che le reazioni cutanee, si manifestano più frequentemente sulle parti esposte e con variabilità stagionale, suggerendo un possibile effetto di fotosensibilizzazione.

I fattori che risultano statisticamente significativi per l'insorgenza di reazioni tardive sono la presenza di allergie, precedenti somministrazioni di MdC, l'appartenenza al sesso femminile e la presenza di malattie concomitanti (Bartolucci *et al*, 2000; Munechika, 2003).

Fattori di rischio

L'età dei pazienti si è dimostrata essere un fattore di rischio. Secondo uno studio di Morcos S. (2001) i pazienti più a rischio sono quelli molto giovani e quelli molto anziani. Invece secondo Munechika (2003) l'incidenza delle reazioni avverse si riduce di 0,984 per ogni aumento di un anno di età del paziente; pertanto sono più frequenti nei pazienti più giovani.

Anche il sesso rappresenta un fattore di rischio con una incidenza di 2,5% tra i maschi e di 3,4% nelle femmine.

Di cruciale importanza è apparsa un'accurata valutazione dello stato clinico del paziente: la ridotta funzionalità renale e cardiovascolare rappresentano i veri fattori di rischio.

L'incidenza delle reazioni tardive è molto più alta nei pazienti con una storia di allergie (incidenza del 7,5% nei pazienti allergici contro il 2,5% nei non allergici), in particolare l'atopia e l'allergia ai pollini sembrano aumentare il rischio; infatti l'incidenza delle reazioni avverse, sia immediate che tardive, è più alta nel periodo della pollinosi (Munechika H, 2003).Queste affermazioni suggerirebbero il possibile coinvolgimento dell'allergia nella patogenesi delle reazioni ai MdC.

Un dato senza dubbio significativo è l'aumentata probabilità di avere reazioni avverse in quei pazienti che già in precedenza hanno manifestato intolleranza ai MdC: in particolare, risulta aumentato di un fattore quattro il rischio di una reazione avversa grave.

Di fondamentale importanza, dunque è un'anamnesi accurata volta ad individuare:

— allergie di grado severo ed in particolare precedenti episodi di reazioni ai MdC;

— stati gravi di sofferenza miocardica ed insufficienza cardiocircolatoria;

— insufficienza renale.

Dosaggio

Benché lo sviluppo di metodiche digitali abbia consentito una drastica riduzione dei volumi di MdC somministrati, tuttavia alcune indagini comportano ancora un carico piuttosto considerevole di agente contrastografico. È ad esempio il caso delle procedure di radiologia interventistica o della TC nelle quali la dose di MdC somministrato può essere, in rari casi, sufficiente a determinare reazioni chemiotossiche. Ne è esemplificativa la necrosi tubulare acuta (NTA) evidenziabile alla TC per la caratteristica intensa opacizzazione del parenchima renale, ma non del tratto escretorio; ciò a dimostrazione della conservata funzione glomerulare, ma della perdita della pervietà tubulare.

In un paziente adulto di circa 70 kg il migliore enhancement epatico in TC addominale si ottiene somministrando 650 mg I/kg corrispondente a circa 45-48 g di I, contenuti in 130 ml di MdC a 350 mg I/ml. Tuttavia dosi di 35-38 g appaiono già soddisfacenti e ragionevoli.

Nello stesso soggetto in un'urografia endovenosa possono essere utilizzati 300 mg I/kg, corrispondenti a 21 g di I.

In ambito pediatrico può invece essere considerata indicativa la dose di 2 ml/kg di MdC a concentrazione di 300 mg I/ml sia per urografia che in TC.

Nella pratica radiologica si può considerare come quantità iniettabile in un adulto 1000 mg I/kg (circa 200 ml di MdC con concentrazione di 350 mg/ml). La quantità può salire, sempre in pazienti con funzionalità renale normale, fino a 1700 mg I/kg come valore massimo non superabile (400 ml di MdC a 300 mg I/ml come indicato da Stacul nella nota riassuntiva (2003). L'insufficienza renale richiede di ridurre drasticamente queste dosi massimali.

Sperimentalmente la dose letale (LD50) per gli HOCA è risultata di 5-10 g I/kg. I LOCA hanno una LD50 pari a circa due–tre volte quella degli HOCA avendo un carico osmolale più basso e contenendo solo piccole quantità di sodio. I dimeri non ionici offrono un margine di sicurezza ancora maggiore, a parte la neurotossicità.

Interazioni farmacologiche

Diverse sono state le segnalazioni in letteratura di interazioni fra farmaci e MdC, soprattutto con i MdC ionici. Queste riguardavano la precipitazione del farmaco (papaverina, protamina, cimetidina, etc.), l'effetto sinergico aritmogeno del verapamil e dei derivati della digitale in cardioangiografia, l'effetto epilettogeno della clorpromazina in mielografia, etc.

Tuttavia alcuni di questi effetti, evidenziati allo stato sperimentale, non hanno trovato conferma clinica mentre altri non hanno più avuto rilievo per l'abbandono dei MdC ionici.

Allo stato attuale solo 3 farmaci meritano menzione (tab. VIII): le biguanidi, i β-bloccanti e l'interleukina-2; solo per quest'ultima, utilizzata nella chemioterapia è stata segnalata una maggiore incidenza di reazioni ritardate.

Le biguanidi (metformina e fenformina) sono antidiabetici orali che trovano indicazioni nei soggetti obesi e iperlipidemici o in associazione con le sulfoniluree. Sono controindicate nelle condizioni che favoriscono l'ipossia tissutale o che riducono la loro eliminazione come l'insufficenza renale. Questi farmaci infatti hanno un meccanismo d'azione multiplo, in particolare stimolano la glicolisi anaerobica con conseguente aumento della produzione di acido lattico (Thomsen H.S., 1999).

TABELLA VIII. — Sintesi delle interazioni tra MdC e trattamento contemporaneo con farmaci.

MdC e farmaci
- Interleukina 2: aumento reazioni ritardate
- β-bloccanti: diminuita efficacia manovre per ipotensione
- Metformina: acidosi lattica in IR

Un aumento dell'insorgenza di acidosi lattica è stato segnalato in seguito a contemporanea somministrazione del MdC, soprattutto con la fenformina che è stata quindi ritirata dal mercato.

Tale effetto non è dovuto all'interazione fra i due principi, farmaco e MdC, ma alla slatentizzazione della nefropatia indotta dal MdC.

Come è stato recentemente evidenziato infatti in questi pazienti l'acidosi lattica ha sempre quale substrato una ridotta funzione renale.

Gli autori suggeriscono perciò di modificare le indicazioni del Royal College of Radiologist che proponeva la sospensione del farmaco nelle 48 precedenti e successive all'indagine contrastografica, spostando l'attenzione su una scrupolosa indagine anamnestica della funzione renale, eventualmente completata con indagini di laboratorio.

La sospensione del farmaco ed uno stretto controllo della funzione renale successiva all'uso del MdC si renderebbe quindi necessaria solo in pochi casi selezionati.

Infine, in pazienti che assumono farmaci β-bloccanti, le manovre adottate per ripristinare i valori pressori in corso di esami contrastografici, sono necessariamente meno efficaci per una ridotta risposta all'adrenalina, senza poter invocare una reale interazione farmacologica tra agente contrastografico e ß-bloccante.Infatti deve essere tenuto presente che con il sistema beta bloccato gli effetti alfa sono presenti o aumentati sicchè l' effetto paradosso è che un eccesso di trattamento, ulteriormente innalza la pressione arteriosa (effetto alfa), e può essere pericoloso. Piuttosto che un dimezzamento più sicuro di dose di farmaco il miglior compromesso è quello basato sulla esperienza o sulla osservazione continuata.

Nefropatia e farmaci

La nefropatia da MdC nella forma più grave è sostenuta dalla necrosi tubulare acuta. I precipitati proteici e il distacco delle cellule tubulari, bloccano i tubuli renali distali e sono responsabili della persistenza del MdC nell'ultrafiltrato, visibili radiologicamente come persistenza del nefrogramma renale per alcuni giorni con reni modicamente ingranditi per l'edema, senza dimostrazione dell'urina opaca nelle vie escretrici per l'arresto della diuresi. Il MdC non ancora filtrato è secreto con la bile per il fenomeno compensativo dell'emuntorio epatico. Tale grave compromissione, piuttosto rara, generalmente non induce un danno permanente ed entro 5-6 giorni la diuresi si ripristina.

Nelle osservazioni recenti tuttavia la maggior parte dei casi si presentano con oliguria e con nefrogramma persi-

stente per 24-48 ore, elevazione della creatininemia con picco dopo 3-5 giorni.

L'analisi dell'urina dimostra: ridotta escrezione di sodio (nella oliguria), scarsa proteinuria, cellule dell'epitelio tubulare, aggregati granulari e sedimenti amorfi. La filtrazione glomerulare ritorna nella norma in 7-10 giorni

Come dimostrato da vari studi le misure più efficaci per ridurre la severità della nefropatia da contrasto sono rappresentate dalla scelta di un MdC non ionico a bassa osmolalità e dall'espansione del volume extracellulare ottenuta mediante infusione di soluzione salina (Allaqaband S, 2002). Un recente studio ha dimostrato che la nefropatia associata all'uso dei mezzi di contrasto era significativamente ridotta con la somministrazione di soluzione isotonica (0,9% NaCl) rispetto alla soluzione ipotonica (0,45% NaCl) (Mueller C., 2002).

Particolare attenzione deve essere rivolta ai pazienti in trattamento con ACE inibitori, FANS, aminoglucosidi e derivati del platino, per l'effetto nefrotossico di tali farmaci.

Partendo dall'osservazione che le specie reattive dell'ossigeno sono coinvolte nella patogenesi della nefrotossicità, la somministrazione di N-Acetilcisteina (NAC), un antiossidante che ha effetto scavenger sui radicali dell'ossigeno ed inibisce la sintesi delle proteine e delle citochine potenzialmente dannose, è stato oggetto di numerosi studi. Tra questi, quello di Tepel (2000) ha dimostrato che la somministrazione profilattica di NAC (600 mg due volte al giorno, il giorno prima ed il giorno stesso dell'esame) ed una adeguata idratazione (NaCl 0,45%) possono prevenire la riduzione della funzione renale indotta dal MdC, nei pazienti con insufficienza renale cronica.

L'efficacia dell'Acetilcisteina si fonderebbe secondo questo studio, oltre che sulla sua capacità antiossidante anche sulla sua capacità di indurre vasodilatazione, contrastando le possibili alterazioni dell'emodinamica renale indotte dal MdC. L'entusiasmo ha indotto a proporre la somministrazione di acetilcisteina quale prevenzione del danno nefrotossico (Morcos, 1999). Tuttavia tre altri studi (Allaqaband, Durham, Briguori, 2002) non hanno confermato questo vantaggio e l'utilizzazione sistematica dell'acetilcisteina non sembra giustificata.

L'efficacia di diuretici come la furosemide, che diminuirebbero il rischio di ischemia midollare, non è ancora stata accertata ed il loro uso routinario non è consigliato. L'attivazione dei recettori della dopamina DA-1 aumenta il flusso renale, ma i risultati ottenuti dall'uso della dopamina sono contrastanti probabilmente in conseguenza della stimolazione non specifica di altri recettori; essa può essere renoprotettiva nei pazienti non diabetici. Risultati più definitivi potranno essere ottenuti da un trial in corso con il fenoldopam, un agonista selettivo dei recettori DA-1 (Stone GW, 2001).

Anche l'adenosina, un vasocostrittore renale, è coinvolta nella patogenesi della nefropatia da contrasto. I risultati ottenuti con teofillina e aminofillina, sono contradditori. Inoltre l'utilità del peptide natriuretico atriale (che aumenta il flusso renale) e degli antagonisti dell'endotelina (un vasocostrittore) rimane ancora da stabilire (Katholi RE, 1995).

Una produzione inadeguata di prostaglandina renale potrebbe essere un fattore patogenetico della nefrotossicità e l'infusione di 20 ng/kg/min di prostaglandina E_1 limita significamene l'incremento di creatinina sierica. Comunque gli effetti sistemici della somministrazione parenterale di prostaglandina E1 come ipotensione e tachicardia ne limitano l'utilizzo clinico (Koch JA, 2000).

Premedicazione

Alla luce delle osservazioni secondo cui le reazioni più severe ai MdC possono essere sostenute da un meccanismo anafilattico uno studio francese suggerisce di sottoporre i pazienti che hanno presentato questo tipo di reazioni ad una accurata investigazione allergologica (skin test) al fine di identificare il MdC responsabile della reazione; tutto questo in modo da evitare una successiva assunzione dello stesso MdC, unica strategia efficace per prevenire ulteriori accidenti allergologici (Dewachter P, 2001).

Non esiste alcuno studio nell'uomo o nell'animale randomizzato, che abbia provato l'efficacia di una profilassi con antistaminici e/o corticosteroidi nel prevenire le reazioni (Marshall GD, 1991; Christiansen, 2002).

Se la premedicazione con 1 o 2 dosi di metilprednisolone (32 mg per os 6-24 ore prima) ha ridotto l'incidenza dei segni minori, essa non ha ridotto l'incidenza delle reazioni più gravi (Lasser, 1994). Le premedicazioni costituiscono una falsa sicurezza ed inoltre bisogna comunque considerare la morbilità propria legata alla prescrizione di questi farmaci.

La sola modalità per assicurare un miglioramento completo ai pazienti che abbiano manifestato una reazione avversa severa è somministrare un trattamento ad hoc nel primo minuto dalla comparsa dei sintomi (Laroche, 1998). Inoltre i radiologi dovrebbero essere in grado di operare un precoce ed efficace trattamento dello shock anafilattico, inclusa la somministrazione di epinefrina.

Aggravamento di patologie pre-esistenti

Bisogna sottolineare come diverse condizioni cliniche , a suo tempo considerate come indicazione esclusiva all'impiego dei MdC a bassa osmolalità, oggi non vengono più considerate tali dagli stessi autori. Esse sono la rinite allergica, le allergie alimentari (purchè di numero e di entità limitate) l'iperazotemia, la gotta, le tireopatie, il mieloma multiplo, il morbo di Waldëstrom e la stessa asma che non appare più come fattore predittivo. [Dato questo peraltro non accettato da molti. Vedi Capitolo Martinelli e Coll.; Tamburrini e Coll. - Nota editor].

Gammopatia monoclonali. — In passato è stato rilevato che nei pazienti affetti da mieloma multiplo, soprattutto nella varietà secernente proteina di Bence–Jones o solo catene leggere libere monoclonali, la somministrazione di MdC potesse causare la precipitazione di queste nel tubulo (myeloma kidney), innescata dalla disidratazione e, forse, parzialmente anche da un effetto diretto del MdC sulle catene polipeptidiche. Studi recenti hanno però evidenziato la bassa incidenza di nefropatia con i LOCA (Barret BJ, 1992, 1993). È quindi opinione attuale che nei pazienti con funzione renale conservata e con adeguata idratazione, il mieloma multiplo non rappresenti una controindicazione alla somministrazione del MdC. Ha comunque perduto significato la ricerca nelle urine della proteina di Bence-Jones.

Anemia falciforme. — La precipitazione dell'emoglobina S con falcizzazione dei globuli rossi sarebbe indotta da variazioni dell'osmolalità conseguenti alla somministrazione di MdC; il maggior rischio si verifica in seguito alla somministrazione intrarteriosa, specie ad alte dosi e in caso di arteriografia cerebrale o cardiaca. Studi in vitro hanno dimostrato un minor rischio di falcizzazione eritrocitaria in seguito all' utilizzo di MdC non ionici.

Feocromocitoma. — Nel passato sono stati segnalati casi di crisi ipertensive anche severe in pazienti con feocromocitoma in seguito a studi vascolari selettivi con iniezione di MdC ionici nella vena o arteria surrenalica dell'organo affetto, e più raramente in TC. Ciò era dovuto al rilascio in circolo di notevoli quantità di catecolamine. Nessuna segnalazione è tuttavia pervenuta con l'uso di MdC a bassa osmolalità.

Tireotossicosi. — In pazienti con uno stato di ipertiroidismo, ancora allo stadio subclinico o già manifesto, la somministrazione di MdC iodati può portare ad uno stato di ipertiroidismo scompensato fino alla crisi tireotossica. Poiché tali alterazioni sono legate all'apporto di iodio, l'incidenza non varia con l'uso dei LOCA. Peculiare è inoltre il fatto che il deterioramento della funzione tiroidea si manifesti non acutamente ma a distanza di settimane o mesi. Utile può essere la somministrazione preventiva di perclorato, che inibisce l'assorbimento dello iodio da parte della tiroide, e di metimazolo che inibisce la sintesi ormonale (Westhoff-Bleck M, 1991). Inoltre l'uso del perclorato appare indicato nei pazienti che devono successivamente essere sottoposti a studio scintigrafico tiroideo, poiché eventuali atomi di iodio libero potrebbero essere captati dalla tiroide con conseguente comparsa di falsi positivi.

Miastenia gravis. — In seguito a somministrazione di MdC iodati diverse sono state le segnalazioni di brevi "crisi" miasteniche. Da segnalare che tutti i pazienti reattivi avevano già in precedenza sintomi bulbari.

Menzione a parte deve essere fatta per lo stato gravidico e l'allattamento: eccezionale è la somministrazione dei MdC in gravidanza per la conseguente esposizione a Rx; in tali casi tuttavia pur non essendo provato l'effetto teratogeno dei MdC, la loro somministrazione dovrebbe essere evitata. Inoltre, nell'ultimo trimestre di gravidanza, in seguito al passaggio del MdC attraverso la placenta potrebbero insorgere disfunzioni della tiroide fetale, quali ipotiroidismo.

Nessuna controindicazione invece all'allattamento dopo somministrazione di MdC: data la scarsa liposolubilità, meno del 1% della dose somministrata alla madre è escreta nel latte materno nelle prime 24 ore. Poichè meno del 1% del MdC ingerito dal neonato viene assorbito nel tratto gastrointestinale la dose assorbita sarà circa lo 0,01% di quella somministrata alla madre. Questa quantità rappresenta meno dell' 1% della dose raccomandata per uno studio contrastografico (ad es. TC) chè è di circa 2 ml/kg.

Secondo le linee guida dell'ACR Committee la madre può continuare regolarmente l'allattamento; tuttavia se la madre appare preoccupata circa i potenziali rischi, può essere consigliata l'astensione dall'allattamento nelle prime 24 ore con l'eliminazione del latte materno in tale intervallo di tempo.

TABELLA IX. — Influenza della gravità della malattia pre-esistente alla comparsa di reazioni al MdC. Classificazione ASA Campus Link, 1993 (56671 pazienti).

	ASA				
	1	2	3	4	5
Pazienti	7942	11126	35004	2510	112
Reazioni	34	572	208	28	3
%	0,42	0,51	0,59	1,11	2,6

Effetti sulla coagulazione

È ormai accertato che i MdC interferiscono con il sistema della coagulazione in particolare inibendo la polimerizzazione della fibrina e l'aggregazione piastrinica e possono potenziare l'azione anticoagulante dell'eparina (Parvez, 1984). Tuttavia tali effetti appaiono meno marcati per i MdC non ionici, specie alle alte concentrazioni. L'effetto anticoagulante è sempre stato considerato come un fattore aggiuntivo protettivo nell'evitare la comparsa di trombi durante le manovre di cateterismo angiografico.

La maggior proprietà anticoagulante dei MdC non ionici è legata alla loro maggiore inerzia e biocompatibilità.

Mentre la maggior parte degli studi sperimentali ha confermato le caratteristiche anticoagulanti dei MdC (Spitzer SG, 2002), alcuni hanno sospettato una loro possibile aumentata attività trombogenica (Pislaru S, 1998). Gli episodi trombotici che si sono verificati durante la somministrazione di MdC indicano la complessa natura del delicato controllo dei meccanismi emostatici e trombotici. I fattori di rischio per le possibili complicanze trombotiche sono rappresentate dalle varie condizioni di ipercoagulabilità come la sindrome da iperviscosità e il deficit di proteina C/S e di antitrombina III (Fareed J, 1990).

Molteplicità dei fattori di rischio

Fra le molteplici cause favorenti o aggravanti il rischio di reazione al MdC deve essere considerata la pre-esistente comorbidità del paziente, più spesso in termini di patologia renale o cardiaca.

L'incidenza di eventi avversi è in diretta proporzione alla severità della malattia sottostante.

In una osservazione prospettica nazionale (Campus Link) di 56671 casi di radiologia diagnostica invasiva e interventistica, la percentuale non solo delle complicanze, ma anche delle reazioni avverse è direttamente proporzionale alla classe di gravità clinica valutata nella scala ASA (1 normale; 5 massima gravità).

Come si può osservare in tabella IX, tanto più grave è lo stato clinico del paziente, tanto più aumenta non solo la gravità, ma anche la frequenza delle reazioni avverse.

Si noti che per classificare lo stato clinico del paziente la scala ASA (American Society of Anesthesiologist) non utilizza esami di laboratorio o altri test, ma solo la valutazione clinica o anamnestica del paziente. Cioè quanto indicato nella circolare del Ministero della Sanità nel 1997, con la

quale si è tolto alla batteria degli esami preliminari la capacità di poter prevenire incidenti da MdC (fig. 8), ribaltando sulla valutazione clinico-anamnestica la più importante prevenzione delle reazioni avverse.

Mezzi di contrasto contenenti gadolinio per esami radiografici

I MdC contenenti gadolinio determinano un enhancement tissutale e vascolare in TC e possono essere usati in alternativa ai MdC contenenti iodio.

In particolare è stato suggerito l'utilizzo dei MdC a base di gadolinio per gli esami radiografici dei pazienti con insufficienza renale, con precedente reazione severa generalizzata ai MdC o sottoposti a trattamento con radioiodio per patologie tiroidee.

Il gadolinio ha un alto numero atomico (Z=64) rispetto allo iodio (Z=53) e un più elevato K edge (50 KeV rispetto a 33 KeV dello iodio). Pertanto il gadolinio assorbe una maggior frazione dello spettro energetico e determina una maggior attenuazione dei raggi X, durante l'acquisizione delle immagini TC.

Alla concentrazione equimolare di 0,5 mol/L l'attenuazione del MdC contenente gadolinio (3069 UH) era approssimativamente del 50% superiore a quella ottenuta con 320 mg/ml del MdC iodato (1979 UH) (Gierada DS, 1999).

Il MdC contenente gadolinio produce pertanto un enhancement vascolare e tissutale sostanzialmente sovrapponibile a quello ottenuto con un analogo volume di MdC iodato, presentando inoltre una analoga biodistribuzione ed escrezione, con quest'ultimo.

L'attenuazione di entrambi i tipi di MdC decresce con l'aumento del voltaggio del tubo radiogeno, se questo oscilla tra 80 e 137 KV, ma questo decremento è più pronunciato per lo iodio.

Se misurato a 120 KV il gadolinio è approssimativamente del 40% più efficiente nell'assorbimento dei raggi X rispetto allo iodio ad una concentrazione equivalente.

La maggior attenuazione TC del gadolinio è determinata dall'interazione fotoelettrica dei fotoni a maggiore energia (Schmitz SA, 1995).

Tuttavia una recente revisione della letteratura, in accordo con i dati sperimentali sugli animali, in merito all'utilizzo di questi MdC non ha indicato una loro minore nefrotossicità rispetto ai MdC iodati (utilizzati a dosi equivalenti per ottenere la stessa attenuazione dei raggi X). Pertanto anche i MdC a base di gadolinio non dovrebbero essere usati nei pazienti con insufficienza renale.

Inoltre anche l'impiego nelle altre due indicazioni (precedenti reazioni severe ai MdC iodati e tireopatia) al dosaggio di 0,3 mmol/kg, non fornisce nella maggior parte dei casi informazioni diagnostiche adeguate (Thomsen HS, 2002).

Linee guida: domande e risposte

Vedi anche Dawson P, Clauß W (1994).

— Una storia di ipersensibilità ai MdC o di allergie può aumentare i rischi dei pazienti sottoposti ad esami con MdC?

I MdC radiografici (così come gli anestetici locali o intravascolari ed i sostituti plasmatici) possono determinare delle reazioni pseudoallergiche. Tuttavia esse hanno raramente una origine immunologica e sono pertanto chiamate pseudoanafilattiche o anafilattoidi.

La loro patogenesi non è nota, ma sembra comprendere l'attivazione del complemento, il rilascio diretto di mediatori, come l'istamina e la serotonina, l'interazione con i sistemi della coaugulazione e fibrinolitici e con il sistema callicreina-chinina.

Lo studio di Katayama ha dimostrato una frequenza di reazioni avverse cinque volte maggiore nei pazienti con pregressa reazione ai MdC (con i MdC non ionici la frequenza era di 11,3% per i soggetti con precedenti reazioni, contro il 2,2% dei pazienti con anamnesi negativa).

Successivi studi eseguiti su campioni più ampi hanno confermato i risultati ottenuti da Katayama, ed hanno affermato anche il minor rischio potenziale dei MdC non ionici nello scatenare reazioni anafilattoidi (Gerstmann, 1991).

— La presenza di diabete mellito rappresenta un rischio nello somministrazione di MdC?

Nei pazienti affetti da nefropatia diabetica la somministrazione di MdC può costituire un alto fattore di rischio di deterioramento della funzionalità renale fino alla comparsa di una franca insufficienza renale acuta.

Tuttavia la comparsa di sclerosi glomerulare e di metaplasia tubulo-interstiziale si verifica raramente, coinvolgendo meno del 50% dei pazienti dopo più di 10 anni di malattia. Pertanto il diabete mellito non rappresenta un fattore di rischio nella somministrazione di MdC iodati.

— Le gammopatie monoclonali rappresentano un rischio nella somministrazione di MdC?

Alcuni anni fa in seguito all'occasionale osservazione in vitro di una aggregazione tra il MdC e le proteine di Bence-Jones si ipotizzò che questa potesse essere la spiegazione degli isolati casi di insufficienza renale acuta osservati nei pazienti affetti da gammopatie monoclonali sottoposti ad urografia. L'ipotesi era che i precipitati paraproteici potessero determinare una nefropatia ostruttiva con conseguente insufficienza renale.

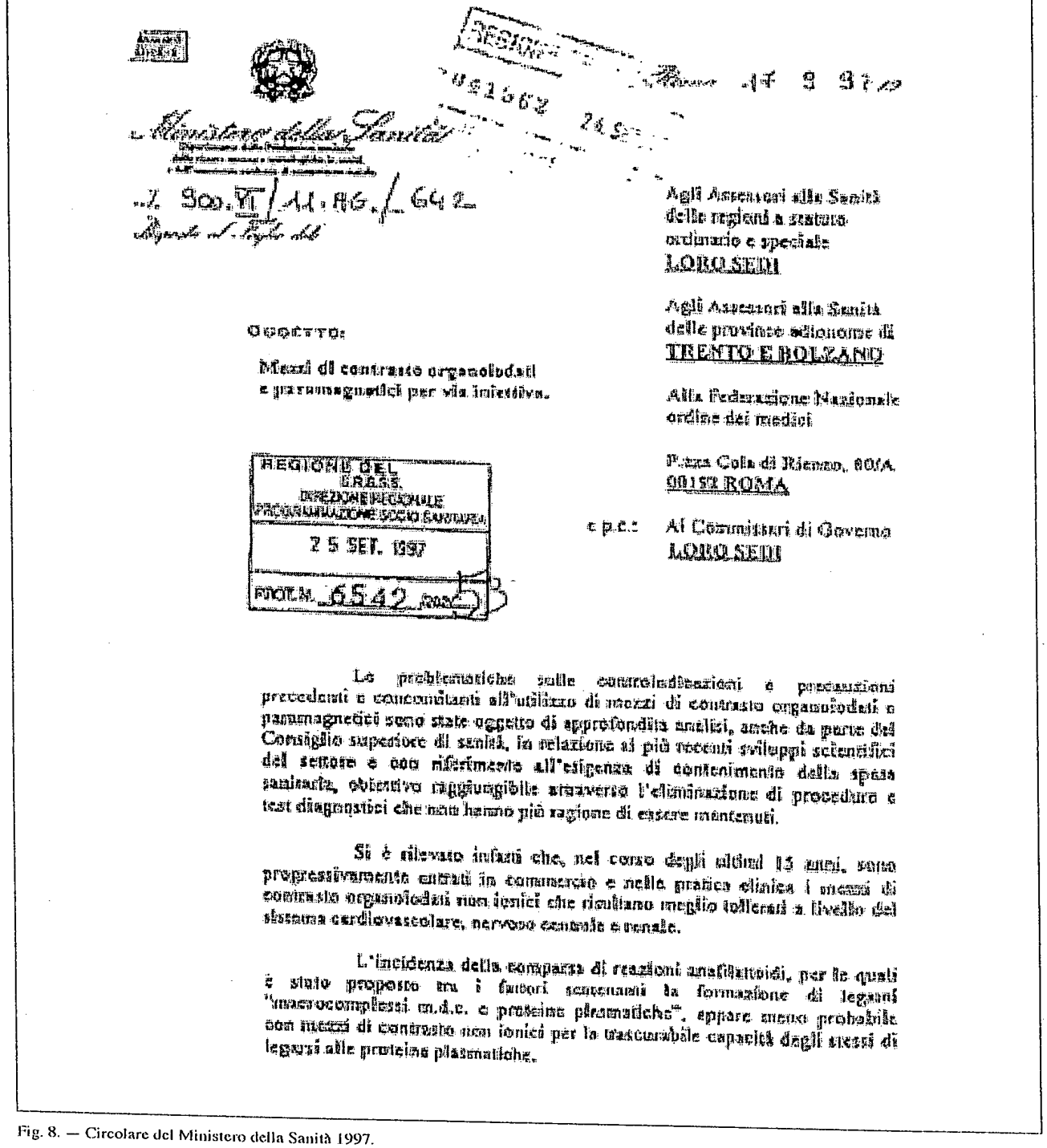
I risultati degli studi *in vivo* non hanno confermato queste ipotesi: recenti studi retrospettivi eseguiti su pazienti affetti da mieloma multiplo indicano che non vi sono influenze sulla escrezione renale.

Pertanto la diagnosi di mieloma multiplo non rappresenta una controindicazione assoluta all'uso dei MdC.

Tuttavia in tali pazienti si raccomanda l'uso dei MdC non ionici associato ad una adeguata idratazione (1000-1500 ml di soluzione fisiologica allo 0,9%), l'utilizzo di una dose di contrasto più bassa possibile e la sospensione dei farmaci nefrotosssici.

— I MdC possono alterare la funzionalità tiroidea?

I MdC iodati non hanno una diretta influenza sulla funzione della tiroide, ma le preparazioni possono contenere piccole quantità di iodio libero. Sia la quota di iodio libero, sia la quota legata possono prendere parte al metabolismo dello iodio e influire così sulla funzione tiroidea.



Ministero della Sanità

DIREZIONE GENERALE DEI SERVIZI FARMACEUTICI

N. 300.VI/11.AG./642

Agli Assessori alla Sanità
delle regioni a statuto
ordinario e speciale
LORO SEDI

Agli Assessori alla Sanità
delle province autonome di
TRENTO E BOLZANO

Alla Federazione Nazionale
ordine dei medici
Piazza Cola di Rienzo, 80/A
00192 ROMA

c.p.c.: Ai Commissari di Governo
LORO SEDI

Oggetto:

Mezzi di contrasto organoiodati
e paramagnetici per via iniettiva.

REGIONE DEL VENETO
DIREZIONE REGIONALE
PROGRAMMAZIONE SOCIO SANITARIA
25 SET. 1997
PROT. N. 6542

La problematiche sulle controindicazioni e precauzioni precedenti e concomitanti all'utilizzo di mezzi di contrasto organoiodati e paramagnetici sono state oggetto di approfondita analisi, anche da parte del Consiglio superiore di sanità, in relazione ai più recenti sviluppi scientifici del settore e con riferimento all'esigenza di contenimento della spesa sanitaria, obiettivo raggiungibile attraverso l'eliminazione di procedure e test diagnostici che non hanno più ragione di essere mantenuti.

Si è rilevato infatti che, nel corso degli ultimi 15 anni, sono progressivamente entrati in commercio e nella pratica clinica i mezzi di contrasto organoiodati non ionici che risultano meglio tollerati a livello del sistema cardiovascolare, nervoso centrale e renale.

L'incidenza della comparsa di reazioni anafilattoidi, per le quali è stato proposto tra i fattori scatenanti la formazione di legami "macrocomplessi m.d.c. e proteine plasmatiche", appare meno probabile con mezzi di contrasto non ionici per la trascurabile capacità degli stessi di legarsi alle proteine plasmatiche.

Fig. 8. — Circolare del Ministero della Sanità 1997.

I mezzi di contrasto non ionici sono oggi utilizzati in oltre il 95% delle indagini urografiche, angiografiche e di tomografia computerizzata.

I prodotti esa-iodati per uso colangiografico, per i quali si aveva maggiore incidenza di reazioni collaterali di tipo medio-grave, non sono più commercializzati a partire dall'anno 1995.

A seguito di questo radicale mutamento del settore è possibile configurare le seguenti tre situazioni meritevoli di considerazione:

1) impiego di mezzi di contrasto di tipo ionico. Nell'utilizzo di tali mezzi di contrasto vanno adottate norme prudenziali, data la minore tollerabilità che caratterizza questi prodotti. Le norme prudenziali non vanno intese come ricorso acritico a vaste batterie di esami di laboratorio, ma come attenta analisi delle condizioni dei pazienti su base clinica ed anamnestica (pazienti a comprovato rischio allergico, pazienti portatori di gravi forme di insufficienza epatica o renale o cardiovascolare o di paraproteinemia di Waldenstrom o di mieloma multiplo);

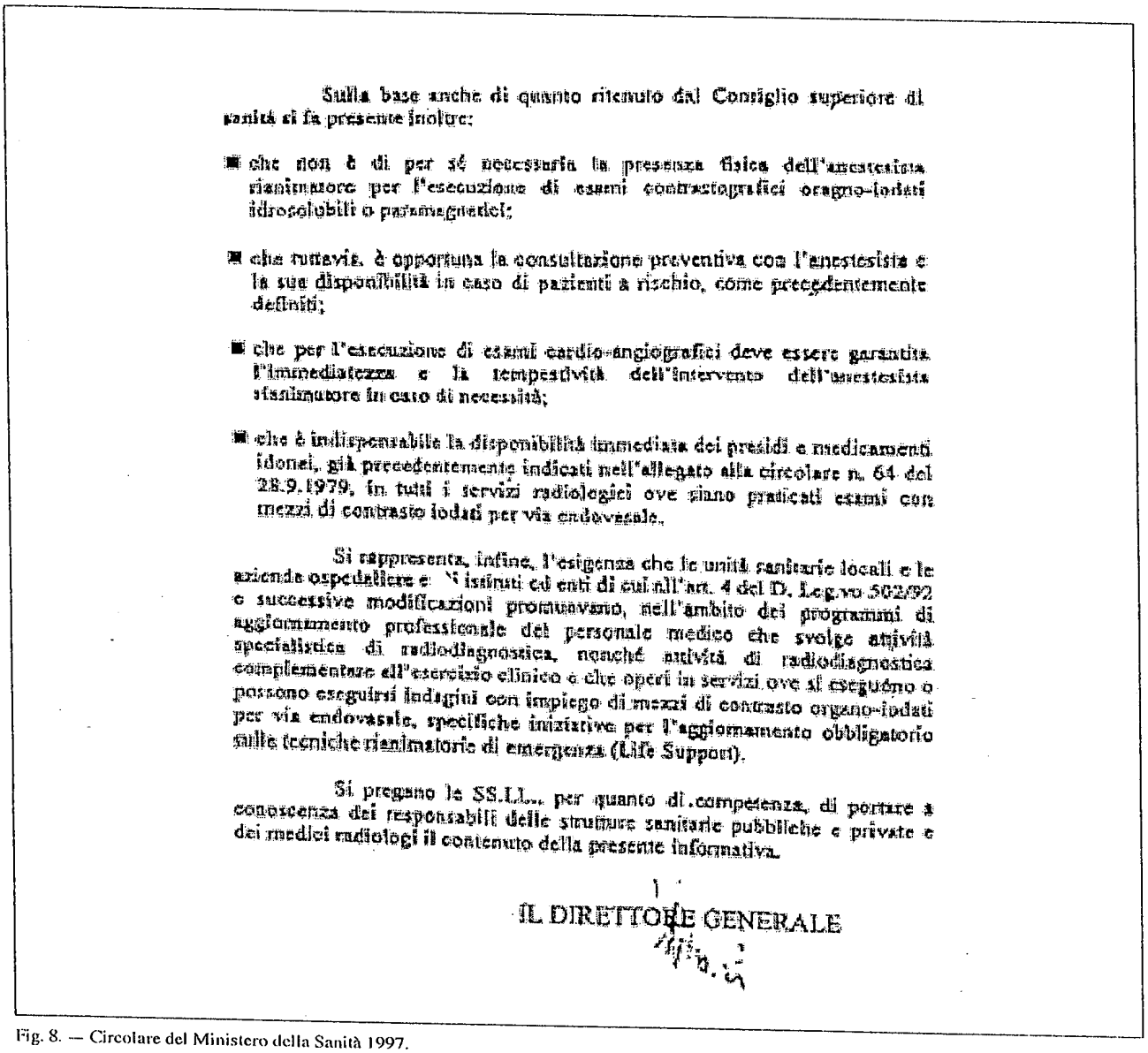
2) impiego di mezzi di contrasto di tipo non ionico. Tali mezzi di contrasto presentano una tollerabilità molto elevata, dimostrata da una larghissima esperienza internazionale. Anche in questo caso, tuttavia, sono da considerare a rischio potenziale i pazienti con gravi forme di insufficienza epatica o renale o cardiovascolare o con paraproteinemia di Waldenstrom o con mieloma multiplo. Tali casi sono da valutare di concerto tra radiologo e medico curante;

3) impiego di mezzi di contrasto paramagnetici. Tali mezzi di contrasto sono una categoria di farmaci totalmente differenti rispetto alle precedenti. I piccoli volumi iniettati e le diverse caratteristiche farmacologiche permettono tranquillità di impiego, pur tenendo in considerazione i rischi generici di ipersensibilità, caratteristici di ogni formulazione iniettabile.

Tenuto conto delle suesposte situazioni si ritiene:

- che la valutazione clinico-anamnestica di ciascun paziente da sottoporre ad indagine con mezzi di contrasto da parte del medico curante, che richiede l'esame, e da parte del radiologo, che esegue l'esame, rappresenti per la prevenzione il momento più importante;
- che il ricorso abituale, in ogni paziente, a batterie di esami/procedure diagnostiche pre-definite, non abbia indicazione ai fini di prevenire incidenti da mezzi di contrasto. Test laboratoristici o procedure diagnostiche sono indicati per definire nei pazienti a rischio il grado delle condizioni patologiche di cui ai punti 1) o 2).

Fig. 8. — Circolare del Ministero della Sanità 1997.



Sulla base anche di quanto ritenuto dal Consiglio superiore di sanità si fa presente inoltre:

- che non è di per sé necessaria la presenza fisica dell'anestesista rianimatore per l'esecuzione di esami contrastografici organo-iodati idrosolubili o paramagnetici;
- che tuttavia, è opportuna la consultazione preventiva con l'anestesista e la sua disponibilità in caso di pazienti a rischio, come precedentemente definiti;
- che per l'esecuzione di esami cardio-angiografici deve essere garantita l'immediatezza e la tempestività dell'intervento dell'anestesista rianimatore in caso di necessità;
- che è indispensabile la disponibilità immediata dei presidi e medicamenti idonei, già precedentemente indicati nell'allegato alla circolare n. 64 del 28.9.1979, in tutti i servizi radiologici ove siano praticati esami con mezzi di contrasto iodati per via endovenosa.

Si rappresenta, infine, l'esigenza che le unità sanitarie locali e le aziende ospedaliere e gli istituti ed enti di cui all'art. 4 del D. Lgs.vo 502/92 e successive modificazioni promuovano, nell'ambito dei programmi di aggiornamento professionale del personale medico che svolge attività specialistica di radiodiagnostica, nonché attività di radiodiagnostica complementare all'esercizio clinico e che operi in servizi ove si eseguono o possono eseguirsi indagini con impiego di mezzi di contrasto organo-iodati per via endovasale, specifiche iniziative per l'aggiornamento obbligatorio sulle tecniche rianimatorie di emergenza (Life Support).

Si pregano le SS.LL., per quanto di competenza, di portare a conoscenza dei responsabili delle strutture sanitarie pubbliche e private e dei medici radiologi il contenuto della presente informativa.

IL DIRETTORE GENERALE

Fig. 8. — Circolare del Ministero della Sanità 1997.

In una condizione di ipertiroidismo latente la quantità di iodio somministrata può essere sufficiente a scatenare un ipertiroidismo clinicamente evidente. Gli effetti sono tuttavia transitori e reversibili.

Nei pazienti ipertiroidei, allo scopo di evitare una esacerbazione dei sintomi con la comparsa di una crisi tireotossica, è necessaria la somministrazione della abituale terapia prima dell'esame con MdC.

Non ci sono test per riconoscere il rischio di una crisi tireotossica.

L'incidenza delle crisi è tuttavia stimata intorno a 1:50000 in Germania.

— Il MdC è un fattore di rischio per i pazienti affetti da gozzo multinodulare?

La somministrazione di iodio aggiuntivo contenuto nel MdC può determinare una transitoria condizione di ipertiroidismo metabolico, per la produzione di ormoni da parte di gruppi di tireociti non più sottoposti al normale feedback.

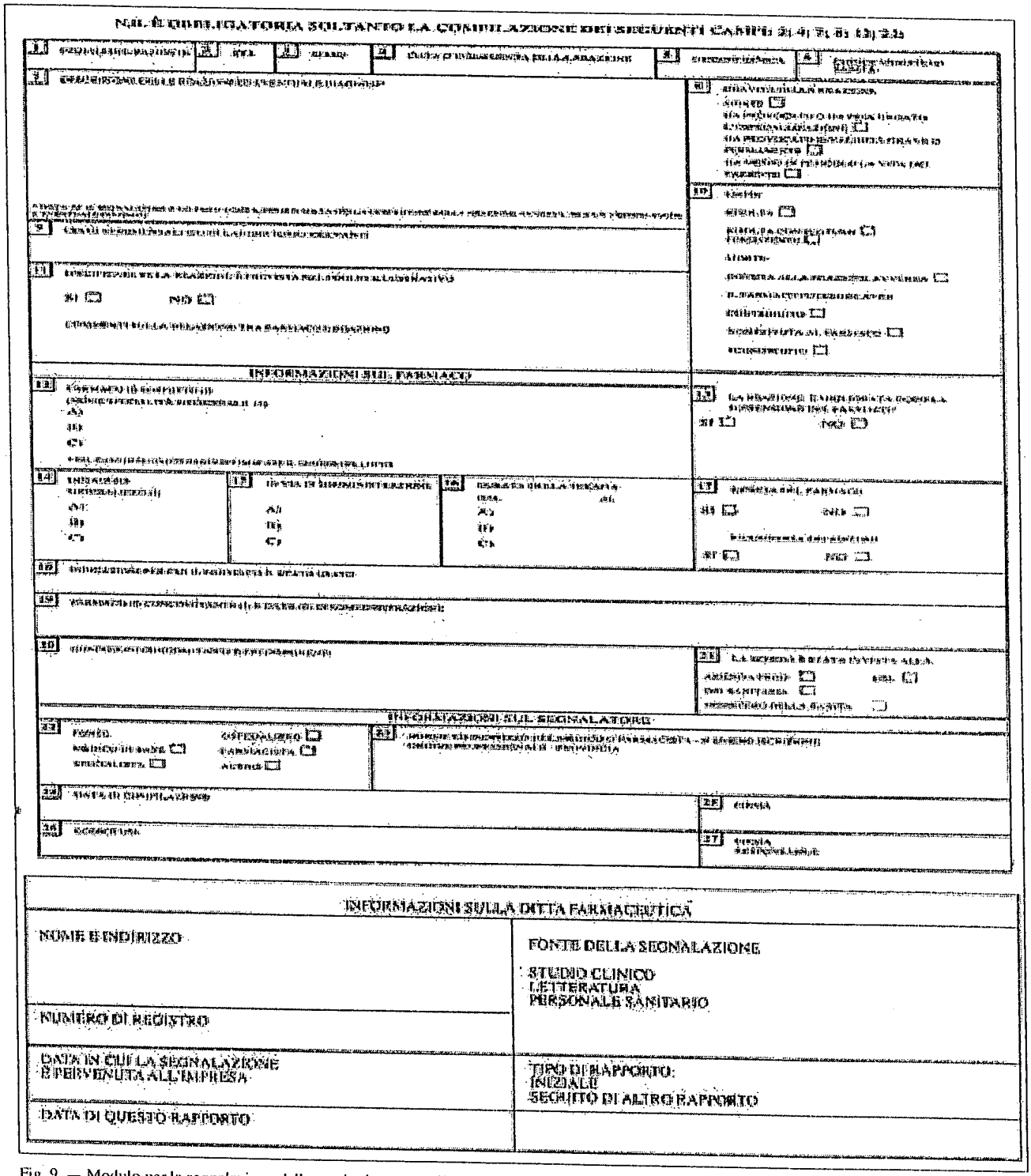


Fig. 9. — Modulo per la segnalazione delle reazioni avverse alla Autorità Sanitaria della Struttura.

Tale condizione si può verificare fino alla completa escrezione dello iodio somministrato con il MdC.

— Qual è l'utilità dei test preliminari per valutare gli eventuali rischi legati all'esposizione ai MdC?

Non esistono esami in grado di prevedere l'insorgenza di effetti secondari alla somministrazione di MdC. In passato era di uso comune la somministrazione di piccole dosi di MdC per via sottocutanea, intradermica o intravasale allo scopo di identificare eventuali reazioni di ipersensibilità.

Spesso i risultati erano dubbi: una risposta positiva al test di ipersensibilità poteva essere seguita da una tolleranza completamente asintomatica al MdC, mentre si potevano verificare reazioni anche gravi dopo un test negativo

L'incertezza interpretativa dei test preliminari, unita all'evidenza che piccole dosi di MdC potevano innescare severe reazioni anafilattoidi indusse nel 1967 il Congresso dei Radiologi Europei a bloccare la somministrazione dei test preliminari.

Oggi è assunto che le reazioni antigene-anticorpo che si verificano dei test preliminari non sono coinvolte nelle reazioni di ipersensibilità ai MdC e che un rapido intervento terapeutico rimane sempre la procedura più importante nel caso in cui si verifichino le suddette reazioni.

— Qual è l'utilità dell'anestesia nel prevenire la comparsa delle reazioni da MdC?

Le caratteristiche stesse dell'anestesia generale impediscono il manifestarsi delle reazioni da MdC moderate come nausea e vomito.

Per quanto riguarda le reazioni cutanee o l'ipotensione non si evidenziano significative differenze nella loro frequenza.

Inoltre le reazioni più severe, come lo shock anafilattico, sono state osservate anche in caso anestesia generale del paziente. Pertanto la sedazione non assicura una assoluta protezione contro le reazioni avverse da MdC e non vi sono giustificazioni al suo utilizzo a scopo precauzionale.

— Che ruolo svolgono gli additivi nella formulazione dei MdC?

I MdC non ionici non contengono citrato di sodio, ma una preparazione di EDTA chiamata calcium disodium edetato. Né questi additivi, né le molecole non ioniche stesse legano significativamente il calcio e ciò contribuisce indubbiamente a ridurre la loro cardiotossicità.

Alcuni studi sugli animali hanno suggerito che l'assenza di ioni sodio nei MdC non ionici potrebbe determinare un aumento nell'incidenza della fibrillazione ventricolare. Nonostante studi successivi abbiano smentito questa ipotesi, è stata suggerita l'aggiunta di sodio sottoforma di sodio citrato.

Ciò allo scopo non solo di supplire alla mancanza di ioni sodio, per minimizzare l'incidenza della fibrillazione ventricolare, ma anche per ripristinare, mediante un efficace legame con il calcio, i potenti effetti anticoaugulanti che erano propri dei MdC ionici.

— I MdC possono essere diluiti o mescolati con altri farmaci?

Le sostanze con cui preferenzialmente si possono diluire i MdC sono la soluzione fisiologica o eventualmente, tenendo conto della particolare osmolalità, l'acqua.

La compatibilità con altre sostanze deve essere giudicata in base al colore, alla limpidezza e al pH, quest' ultimo non dovrebbe mai discostare da un range definito (ad esempio nel caso dello Iopromide, Ultravist, questo range oscilla tra 6,5 e 8).

A causa del possibile rilascio di iodio, il mescolamento con altre sostanze può avere forti effetti riducenti.

Anche la creazioni di soluzioni con metalli pesanti dovrebbe essere evitata; il mescolamento con altri farmaci dovrebbe avvenire solo dopo adeguati test di compatibilità e le soluzioni ottenute dovrebbero essere preparate immediatamente prima della somministrazione.

— Esistono atti formali da eseguire in caso di reazione avversa?

Le reazioni avverse osservate non vanno registrate solo nelle cartelle, o file, della Struttura, ma vanno segnalate all'Autorità Sanitaria responsabile della Struttura, in modulo predisposto dal Ministero della Salute. Il modulo (fig. 9) per la segnalazione delle reazioni avverse a farmaci e quindi anche a MdC, deve essere presente in ogni sede ove vengano eseguite queste somministrazioni. Inoltre il Ministero della Salute nel potenziamento della farmacovigilanza ha istituito una Rete Nazionale di Farmacovigilanza in ambiente web. I MdC, farmaci, inclusi nella classe VO della categoria terapeutica ATC (Guidelines for ATC, 2002), sono stati oggetto nell'anno 2001 del 2,4% di tutte le segnalazioni di reazioni avverse ai farmaci (Bollettino d'informazione sui farmaci, 2002), valore pari a quello delle reazioni a penicilline e cefalosporine. La segnalazione di reazione avversa, che va fatta alle Autorità locali sanitarie, in precedenza era obbligatoria per tutte le reazioni, anche lievi e transitorie; purtroppo su questo punto i radiologi non brillarono per correttezza. Molto recentemente l'obbligo di segnalazione è stato limitato alle reazioni avverse gravi o inattese (D.Lgs. 8 aprile 2003 n. 15 del Ministero della Salute) con modalità tempestiva (entro 7 giorni). Va infine ricordato che non è necessaria una dimostrata responsabilità del MdC, ma è sufficiente che l'operatore abbia solo il sospetto della attribuzione al MdC della reazione.

Sintesi delle tappe di esecuzione di un esame con MdC

Il radiologo, o lo specialista che esegua attività radiodiagnostica complementare all'esercizio clinico, quando debbano ricorrere alla somministrazione di MdC per una procedura diagnostica o interventistica, devono tenere conto degli atti sequenziali nella realizzazione della procedura.

1) accettazione dell'indagine richiesta e valutazione della congruità;

2) valutazione clinico-anamnestica del paziente, dei fattori di rischio, anche dopo eventuale consulto (fig. 10) ed esecuzione della procedura;

FRONTE

UNIVERSITÀ/OSPEDALE DI ..... AZIENDA OSPEDALIERA .....

Data .....

MODULO RICHIESTA PER ESAMI RADIOLOGICI CON MEZZI DI CONTRASTO IODATI
(Nota Min. San. 17.09.97)

COGNOME E NOME _____

nato a _____ il _____

residente a _____ via _____ tel. _____

ESAME RICHIESTO _____

INDICAZIONI _____

Per procedere all'esame con mezzo di contrasto iodato ionico/non ionico, sono annotate le seguenti valutazioni clinico-anamnestiche:

- comprovato rischio allergico a contrasti iodati o altre sostanze	SI' ☐	NO ☐
- forme di grave insufficienza epatica o renale o cardiovascolare	SI' ☐	NO ☐
- diabete, paraproteinemie	SI' ☐	NO ☐

Note: (alcool, droghe, farmaci biguanidi, interleukine, β-bloccanti) _____

_____

Il medico richiedente _____

VALUTAZIONE ANAMNESTICA E ACCETTAZIONE ESAME _____

_____

Il radiologo

CONSENSO INFORMATO

Informato dell'indicazione all'indagine e degli eventuali rischi, il paziente dichiara di acconsentire allo svolgimento dell'indagine _____

Il paziente _____

Fig. 10. — Modulo-richiesta per esami radiologici con MdC iodati.

RETRO

INVIO AD ACCERTAMENTI / CONSULTO CON ANESTESISTA

_____

_____

Il radiologo _____

ACCETTAZIONE ESAME SI' ☐ NO ☐

SOSTITUZIONE ESAME PROPOSTO CON ALTRO _____

_____

Il radiologo _____

Fig. 10. — Modulo-richiesta per esami radiologici con MdC iodati.

3) in seguito a partecipazione ai corsi di aggiornamento obbligatorio previsti, capacità di attuare le manovre rianimatorie immediate tese a mantenere le funzioni vitali del paziente in caso di grave reazione avversa (BLS, Basic Life Support; Circolare del Ministero della Sanità, 1997; Fig. 8);

4) predisposizione dei mezzi di intervento: materiali, farmaci, servizi di rianimazione;

5) segnalazione delle reazioni avverse: tale atto professionale deve essere condiviso e rigorosamente attuato anche ai fini medico-legali.

Bibliografia

Allaqaband S *et al*: Prevention of contrast media associated nephropathy: randomized comparison of 2 hydration regimens in 1620 patients undergoing coronary angioplasty. Arch Intern Med 162: 329-336, 2002.

Aspelin P *et al*: Nephrotoxic effects in high-risk patients undergoing angiography. N Engl J Med 348: 491-499, 2003.

Barret BJ *et al*: A comparison of nonionic low-osmolality radiocontrast agents with ionic, high-osmolality during cardiac catheterisation. Engl J Med 326: 431-436, 1992.

Barret BJ, Carlisle EJ: Meta-analysis of the relative nephrotoxicity of high- and low-osmolality iodinate contrast media. Radiology 188: 171-178, 1993.

Bartolucci F *et al*: Reazioni tardive a un mezzo di contrasto radiologico (Iopamidolo-Bracco) Studio prospettico. Radiol Med 100: 273-278, 2000.

Becker J: Evaluation of renal function. Radiology 179: 337-338, 1991.

Berg KJ *et al*: Nephrotoxicity related to RX contrast media. Adv X ray contrast 1: 10-18, 1993.

Bettman MA *et al*: Ionic versus nonionic contrast agents for intravenous use: are all the answers in? Radiology 175: 616-618, 1990.

Bettman MA *et al*: Intravascular contrast agents. Acta Radiol 400: 3-7, 1996.

Bettmann MA *et al*: Adverse events with radiographics contrast agents: results of the SCIVR contrast agent registry. Radiology 203: 611-620, 1997.

Bollettino d'informazione sui farmaci Anno IX: 8-18, 2002.

Briguori C *et al*: Acetylcysteine and contrast agent-associated nephrotoxicity. JACC 40: 298-303, 2002.

Christiansen C *et al*: Delayed allergy-like reactions to X-ray contrast media: mechanistic considerations. Eur Radiol 10: 1965-1975, 2000.

Christiansen C: Late-onset allegy-like reactions to X-ray contrast media. Curr Opin Allergy Clin Immunol 2: 333-339, 2002.

Cochran ST *et al*: Trends in adverse events from iodinated contrast media. Acad Radiol 9 (Suppl 1): S65-S68, 2002.

Cockcroft DW *et al*: Nephron. 16: 31-41, 1976.

Dawson P, Clauß W: Contrast media in practice. Springer-Verlag, Berlin, H., NY, 1994.

Deray G *et al*: Radiocontrast nephrotoxicity (a review). Radiology 30: 221-225, 1995.

Dewachter P *et al*: Severe reactions to iodinated contrast agents: is anaphylaxis responsible? J Radiology 82: 963, 2001.

Durham JD *et al*: A randomized controlled trial of N-acetylcysteine to prevent contrast nephropathy in cardiac angiography. Kidney Int 62: 2202-2207, 2002.

D.Lgs. 8 aprile 2003 n. 95 in G.U. n. 101 del 3 maggio 2003.

FarceDJ *et al*: Thrombogenic potential of non-ionic contrast media? Radiology 174: 321-325, 1990.

Feltrin GP *et al*: Risk factors for serious adverse events in diagnostic cardiovascular procedures. Eur Radiol S261, 1997.

Gerstmann BB: Epidemiologic critique of the report o adverse reactions to ionic and nonionic media by the Japanese Committe on the safety of contrast media. Radiology 178: 787, 1991.

Gierada DS *et al*: Gadolinium as a contrast agent: assesment in a porcine model. Radiology 210: 829-834, 1999.

Guidelines for ATC classification and DDD assignement, 5th ed. WHO Collaborating Centre for Drug Statistics Methodology. Oslo; 2002.

Katayama H *et al*: Adverse reactions to ionic and nonionic contrast media. Radiology 175: 621-628, 1990.

Katholi RE *et al*: Nephrotoxicity from contrast media: attenuation with theophylline. Radiology 195: 17-22, 1995.

Koch JA *et al*: Prostaglandin E1: a new agent for the prevenction of renal dysfunction in high risk patients caused by radiocontrast media? Nephrol Dial Transplant 15: 43-49, 2000.

Kou-Gi S *et al*: Acetylcysteine protects against acute renal damage in patients with abnormal renal function undergoing a coronary procedure. JACC 40: 1383-1388, 2002.

Laroche D *et al*: Anaphylactoid and anaphylactic reactions to iodinated contrast material. Allergy 54, Suppl 58: 13-16, 1999.

Laroche D *et al*: Mechanisms of severe immediated reactions to iodinated contrast media. Radiology 209: 183-190, 1998.

Lasser EC *et al*: Pretreatment with corticosteroids to prevent adverse reactions to nonionic contrast media. AJR 162: 523-526, 1994.

Levin DC *et al*: Coronary arteriography. In: Braunwalfd E (ed): Heart disease. A textbook of cardiovascular medicine, 1st ed, pp. 238-239 Philadelphia: WB Saunders, 1992.

Love L *et al*: The persistent computed tomography nefrogran: its significance in contrast associated nephrotoxicity. Br J Radiol 67: 951-957, 1994.

Mare K *et al*: Contrast media induced pulmonary edema. Comparison of ionic and non-ionic agents in animal model. Invest Radiol 19: 566-569, 1984.

Maqrshall GD *et al*: Comparison of three pre-treatment protocols to prevent anaphylactoid reactions to radiocontrast media. Ann Allergy 67: 70-74, 1991.

Mikkonen R: Acute and late adverse reactions to low-osmolal contrast media. Acta Radiol 36: 72-76, 1995.

Mita H *et al*: Detection of IgE antibody to radiocontrast medium. Allergy 53: 1133-1140, 1998.

Morcos SK: Contrast media induced nephrotoxicity. Br J Radiol 71: 357-365, 1998.

Morcos SK *et al*: Adverse reactions to iodinated contrast media. Eur Radiol 11: 1267-1275: 2001.

Morcos SK *et al*: Prevention of generalized reactions to contrast media: a consensus report and guidelines. Eur Radiol 11: 1720-1728, 2001.

Mueller C *et al*: Prevention of contrast media-associated nephropathy: randomized comparison of 2 hydration regimens in 1620 patients undergoing coronary angioplasty. Arch Intern Med 11: 162: 329-336, 2002.

Munechika H *et al*: A prospective survey of delayed adverse reactions to iohexol in urography and computed tomography. Eur Radiol 13: 185-194, 2003.

Munechika H *et al*: Delayed adverse reactions to nonionic contrast medium (iohexol) in IV use. Acad Radiol 9 (Suppl 1): S69-71, 2002.

Palmer FJ: The RACR survey of intravenous contrast media reactions final report. Australas Radiol 32; 426-428, 1988.

Parvez Z *et al*: Antiplatelet action of intravascular contrast media implications in diagnostic procedures. Invest Radiol 19: 208-211, 1984.

Pislaru S *et al*: *In vivo* effects of contrast media in coronary thrombolysis. J Am Coll Cardiol 32: 1102, 1998.

Schmitz SA *et al*: Evaluation of gadobutrodol in a rabbit model as a new lantanide contrast agent for computer tomography. Invest Radiol 11: 644-649, 1995.

Solomon R *et al*: Contrast-medium-induced acute renal failure. Kidney Int 53: 230-242, 1998.

Speck U: Contrast Media. Overview, Use and Pharmaceutical Aspects. Springer-Verlag, Berlin, H., NY (1999).

Spitzer SG *et al*: Influence of two non-ionic radiographic contrast media with different osmolalities on coagulation in invasive cardiology. Acta Radiol 43: 617-622, 2002.

Stucul F, Cova M: Nefrotossicità da mezzi di contrasto. Quali problemi nella pratica clinica ? Radiol Med 105: 36-41, 2003.

Stone GW *et al*: Design and rationale of Contrast- a prospective, randomized, placebo-controlledtrial of fenoldopan mesylate for the prevention of radiocontrast nephropathy. Rev Cardiovasc Med 2S: 31-36, 2001.

Tepel M *et al*: Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. N Engl J Med 343: 180-184, 2000.

Thomsen HS *et al*: Contrast media and metformin: guidelines to diminish the risk of lactic acidosis in non-insulin-dependent diabetics after administration of contrast media. Eur Radiol 9: 738-740, 1999.

Thomsen HS *et al*: Gadolinium-containing contrast media for radiographic examinations: a position paper. Eur Radiol 12: 2600-2605, 2002.

Westhoff-Bleck M *et al*: The adverse effects of angiographic radiocontrast media. Drug Safety 6: 28-36, 1991.

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ARTICLES

Contrast media-induced nephrotoxicity--questions and answers

SK Morcos
Department of Diagnostic Imaging, Northern General Hospital NHS Trust, Sheffield, UK.

The intravascular administration of **contrast media** (CM) can produce acute haemodynamic changes in the kidney characterized by an increase in renal vascular resistance and a decrease in the glomerular filtration rate (GFR). These changes may lead to clinically significant reduction in renal function in patients with pre-existing risk factors such as diabetic nephropathy, congestive heart failure and dehydration. The pathophysiology of the renal haemodynamic effects of CM involves activation of the tubuloglomerular feedback (TGF) mechanism and the modulation of the intrarenal production of vasoactive **media**tors such as prostaglandins, nitric oxide, endothelin and adenosine. The TGF response is osmolality-dependent and accounts for about 50% of the acute functional effects of high osmolar CM on the kidney. Reduction in the synthesis of the endogenous vasodilators nitric oxide and prostaglandins increases the nephrotoxicity of CM. Endothelin and adenosine play a crucial role in **media**ting the acute functional effects of CM. Antagonists of these **media**tors attenuate the reduction in renal function induced by **contrast** agents. Vacuolization of the cells of the proximal tubules and necrosis of those of the medullary ascending limbs of loops of Henle are the main structural effects of CM in the kidney. The reduction in renal function induced by CM could be minimized by the use of low osmolar CM and adequate hydration. The prophylactic administration of calcium channel blockers and adenosine antagonists such as theophylline may also offer some protective effect.

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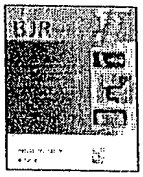
[Abstract] [Full Text] [PDF]



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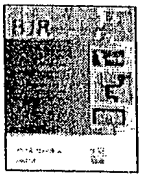

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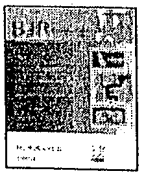

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N. Engl. J. Med., February 6, 2003; 348(6): 491 - 499.

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Radiographic contrast media induced nephropathy: experimental observations and the protective effect of calcium channel blockers

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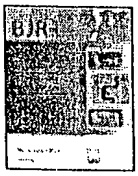

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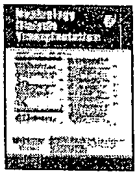

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Full paper

Radiographic contrast media induced nephropathy: experimental observations and the protective effect of calcium channel blockers

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Abstract

Combined acute inhibition of the synthesis of nitric oxide with L-nitroarginine methyl ester (L-NAME) and of prostacycline synthesis with indomethacin predisposes rats to severe renal injury from radiographic **contrast media**. The reliability of this pharmacological manipulation in the study of radiographic **contrast** medium induced nephropathy (RCMN) was investigated. Adult male Sprague–Dawley rats were injected with iv L-NAME (10 mg kg^{-1}) and iv indomethacin (10 mg kg^{-1}) 15 min apart and prior to injection of RCM or normal saline (control group). A dose-dependent reduction in renal function was observed after intravascular injection of the high osmolar RCM diatrizoate (Angiografin, 306 mgI ml^{-1}). A significant ($p<0.01$) increase in serum creatinine (Cr) (from 54.66±8.39 μmol l^{-1} to 171.96±24.49 μmol l^{-1} and from 80.95±6.73 μmol l^{-1} to 204.76±16.73 μmol $^{-1}$, n=5 per group) was observed 24 h after injection of 6 ml and 8 ml of diatrizoate, respectively. The increase in serum Cr after injection of 8 ml of diatrizoate recovered spontaneously to 80.87±8.70 μmol l^{-1} 7 days after injection. No significant change in renal function was observed in the control group (n=5) receiving 8 ml kg^{-1} of normal saline or after injection of 4 ml of diatrizoate (serum Cr 69.84±5.5 μmol l^{-1} pre **contrast** injection and 66.67±13.47 μmol l^{-1} 24 h post **contrast** injection, n=5). The increase in serum Cr observed with 6 ml of diatrizoate was significantly higher ($p<0.01$) than the rise induced by equivolume of the low osmolar non-ionic monomer iopromide (Ultravist, 300 mgI ml^{-1}) (serum CR 68.47±8.39 μmol l^{-1} pre **contrast** injection and 143.59±32.03 μmol l^{-1} 24 h post **contrast** injection, n=5). The calcium channel blocker diltiazem (10 mg kg^{-1} injected intraperitoneally 30 min prior to RCM injection) prevented the rise in serum Cr

observed with 6 ml of diatrizoate (serum Cr pre **contrast** injection 70.31±7.28 μmol^{-1} and 78.21±17.81 μmol^{-1} 24 h post **contrast** injection in animals pre-treated with diltiazem, n=5). The protective effect against RCM-induced reduction in renal function was less with lower doses of diltiazem. In conclusion, the animal model used is reliable and reproduced previously established observations in the field of RCMN. The protective effect of a calcium channel blocker at the appropriate dose against RCMN has also been shown. The clinical effectiveness of this class of drugs in preventing RCMN requires further evaluation.

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Radiographic **contrast** medium (RCM) induced nephropathy (RCMN) remains an important cause of hospital-acquired renal failure [1–3]. The incidence of RCMN is likely to increase with the wide use of spiral CT imaging with intravascular **contrast** enhancement, including CT angiography and perfusion studies, as well as interventional cardiovascular procedures particularly in patients with compromised general health. An understanding of the mechanisms responsible for the renal effects of RCMN has improved with the use of a wide range of experimental animal models [4]. The endogenous biological substances endothelin and adenosine have recently been identified as important **media**tors of the renal effects of radiographic **contrast media** [5,6]. Renal ischaemia appears to be an important prerequisite for the development of RCMN, which is difficult to induce in animals with normal renal function and perfusion. Several experimental animal models have been used in studies of RCMN, including rabbits pre-conditioned by salt depletion and indomethacin [7], rats exposed to multiple insults (uninephrectomy, pre-treatment with indomethacin and salt depletion for several days before RCM administration) [8], rats with hypertension induced by oral L-nitroarginine methyl ester (L-NAME) for several weeks before the administration of RCM [9], dogs with congestive heart failure produced by rapid ventricular pacing [10], aging spontaneously hypertensive male rats [11] and rats with renal ischaemia induced by the combined acute inhibition of the synthesis of the endogenous vasodilators nitric oxide and prostacycline [12]. The latter model is easy to prepare, involves only an acute pharmacological pre-treatment and does not require surgical intervention. In this study we have investigated the reliability of this animal model in the investigation of RCMN. The dose dependency and time course of the renal effects of RCM has also been assessed, as well as comparing the renal tolerance of high osmolar and low osmolar radiographic **contrast media** and the protective effect of a calcium channel blocker against RCMN.

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Experimental protocol

Adult male Sprague–Dawley rats (200–350 g, Shanghai Medical University breed, China) were kept in metabolic cages for 24 h prior to the insults, with free access to tap water and standard rat chow. After a baseline 24-h urine collection, the rats were anaesthetized with intraperitoneal injection of sodium pentobarbital (4 mg per 100 g body weight). The left femoral vein and artery were cannulated and a baseline blood sample (1 ml) was drawn from the femoral vein. 10 mg kg^{-1} indomethacin and 10 mg kg^{-1} L-NAME were administered via the femoral vein at 15 min intervals. After an additional 15 min, RCM or 0.9% normal saline (control)

were injected via the femoral artery cannulation. The rats were kept in the metabolic cages for another 24 h for urine collection, without access to water but freely fed with standard rat chow. At the end of this period rats were anaesthetized with ether gas and a blood sample (1 ml) was drawn, either from the inferior vena cava at laparotomy (in experiments 1, 2 and 4 rats were sacrificed at this stage) or from the femoral vein (experiment 3). The rats were allowed to recover, with free access to tap water and standard rat chow. Blood was sampled 7 days later from the right femoral vein under brief ether anaesthesia (experiment 3).

Serum and urine creatinine (Cr) were determined by standard colorimetric methods using a 721 Spectrophotometer (Shanghai No. 3 Analytical Instrument Factory, Shanghai, China).

(1) Effects of different doses of RCM on renal function

After the injection of indomethacin and L-NAME, rats were injected with either 4 ml kg^{-1}, 6 ml kg^{-1} or 8 ml kg^{-1} of diatrizoate (306 mgI) or 8 ml kg^{-1} of normal saline (control group) via the femoral arterial cannula over a 5 min period (n=5 per group). Serum Cr and Cr clearance were measured 24 h pre and 24 h post injection of diatrizoate or saline, as indicated in the experimental protocol.

(2) Effects of the high osmolar diatrizoate and the low osmolar iopromide on renal function

After injection of standard doses of indomethacin and L-NAME, either 6 ml kg^{-1} of diatrizoate (306 mgI ml^{-1}) or iopromide (300 mgI ml^{-1}) were administered via the femoral arterial cannula over a 5 min period (n=5 per group). Serum Cr and Cr clearance were measured 24 h pre and 24 h post injection of RCM, as indicated in the experimental protocol.

(3) Time course of renal function after injection of diatrizoate

After injection of standard does of indomethacin and L-NAME, 8 ml kg^{-1} (306 mgI ml^{-1}) of diatrizoate were administered via the femoral arterial cannula over a 5 min period (n=5). Serum Cr was measured 24 h before the insults and 24 h after and 7 days after the injection of diatrizoate.

(4) Effect of the calcium antagonist diltiazem in preventing RCMN

After injection of standard doses of indomethacin and L-NAME, an intraperitoneal injection of either 2 mg kg^{-1}, 6 mg kg^{-1} or 10 mg kg^{-1} of diltiazem (4 ml for all three groups with the concentration of dilitiazem being 0.5 mg ml^{-1}, 1.5 mg ml^{-1} and 2.5 mg ml^{-1}, respectively) or 4 ml of normal saline control group was given. 30 min later, 6 ml kg^{-1} of diatrizoate (306 mgI ml^{-1}) was administered via the femoral arterial cannula over a 5 min period (n=5 per group). Serum Cr and Cr clearance were measured 24 h pre and 24 h post injection of diatrizoate, as indicated in the experimental protocol.

Materials

The radiographic **contrast media** used were the high osmolar ionic monomer diatrizoate (Angiographin, 306 mgI ml^{-1}; Schering AG, Berlin, Germany) (osmolality 1500 mosmol kg^{-1} H_2O) and the low osmolar non-ionic monomer iopromide (Ultravist, 300 mgI ml^{-1}; Schering AG, Berlin, Germany) (osmolality 610 mosmol kg^{-1} H_2O). Indomethacin (Sigma Chemical Co., St Louis, MO) was dissolved in phosphate buffer (pH 8–9) at a concentration of 3.3 mg ml^{-1}. L-NAME (L-Nitro-arginine methyl ester) (Sigma Chemical Co. St Louis, MO), was dissolved in 0.9% normal saline at a concentration of 4 mg ml^{-1}. Diltiazem (Shanghai Yan'an Pharmaceutical Factory, Shanghai, China) was dissolved in 0.9% normal saline at concentrations of 0.5 mg ml^{-1} 1.5 mg ml^{-1} and 2.5 mg ml^{-1} respectively.

Analysis of results

All values were reported as mean±standard deviation (SD). Serum Cr levels were presented in μmol l^{-1}. When comparing the magnitude of serum Cr between groups, the changes in percentage, *i.e.* [(serum Cr after insults - serum Cr before insults)/serum Cr before insults] x 100%, were applied. Cr clearance was measured using the formula UV/P, where U=Cr concentration in urine, V=urine volume min^{-1} and P=serum Cr. The percentage change of Cr clearance before and after the insults {[(Cr clearance before insults-Cr clearance after insults)/Cr clearance before insults] x 100%} were presented.

The Student's t-test for paired data was used to determine the significant differences within groups. Analysis of variance was used for comparisons between groups. $p<0.05$ was considered significant.

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Experiment 1

The control group (n=5) receiving 8 ml kg^{-1} of normal saline showed no significant changes in serum Cr or Cr clearance (Figure 1). Animals injected with diatrizoate showed dose-dependent changes in serum Cr and Cr clearance (Figure 1).

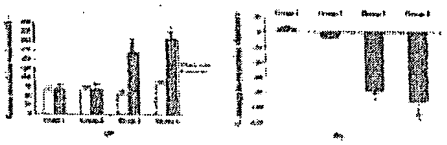

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Figure 1. Effects of different doses of radiographic **contrast** medium on renal function of rats pre-treated with indomethacin and L-nitroarginine methyl ester (L-NAME). (a) Serum creatinine (Cr) before (□) and 24 h after (▨) injection of 8 ml kg^{-1} saline (Group 1, control) or increasing doses of diatrizoate (Group 2, 4 ml kg^{-1}; Group 3, 6 ml kg^{-1}; and Group 4, 8 ml kg^{-1}). Significant increases ($^{*}p<0.01$) in serum Cr occurred in Groups 3 and 4, but no significant rise occurred in Groups 1 and 2 (n=5 per group). The rise in serum Cr in Group 4 was significantly higher ($p<0.05$) than in Group 3. (b) Percentage change in Cr clearance from baseline (24 h pre injection) to 24 h post injection with 8 ml kg^{-1} saline (Group 1, control) or increasing doses of diatrizoate (Group 2, 4 ml kg^{-1}; Group 3, 6 ml kg^{-1}; and Group 4, 8 ml kg^{-1}. Significant decreases ($^{*}p<0.01$) in Cr clearance occurred in Groups 3 and 4 but no significant change occurred in Groups 1 and 2 (n=5 per group). The decrease in Cr clearance in Group 4 was larger ($p<0.05$) than in Group 3.

Experiment 2

The effects of the ionic RCM diatrizoate on renal function were more severe than those of the non-ionic RCM iopromide (Figure 2).

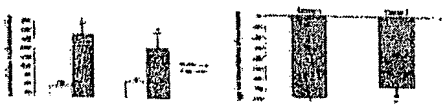
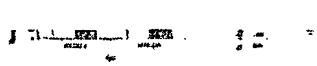


Figure 2. Effects of the high osmolar diatrizoate and low osmolar iopromide on renal function of rats pre-treated with indomethacin and L-nitroarginine methyl ester (L-NAME). (a)



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Serum creatinine (Cr) before (□) and 24 h after (▨) injection of either 6 ml kg^{-1} of diatrizoate (Group 1) or iopromide (Group 2). Significant increases ($^{*}p<0.01$) in serum Cr occurred in both groups, but the rise in Group 1 was significantly higher ($p<0.05$) than in Group 2 ($n=5$ per group). (b) Percentage in Cr clearance from baseline (24 h pre injection) to 24 h post injection of 6 ml kg^{-1} of diatrizoate (Group 1) or iopromide (Group 2). Significant decreases ($^{*}p<0.01$) in Cr clearance occurred in both groups, but the reduction in Group 1 was more severe ($p<0.05$) than in Group 2 ($n=5$ per group).

Experiment 3

The rise in serum Cr induced by 8 ml kg^{-1} of diatrizoate at 24 h post **contrast** injection recovered spontaneously at 7 days (Figure 3).

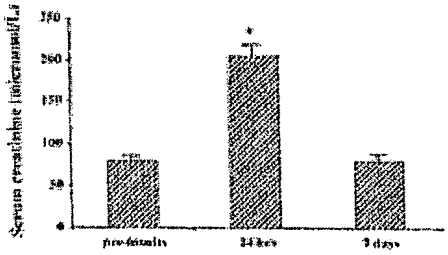

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Figure 3. Time course of renal function after injection of diatrizoate in rats pre-treated with indomethacin and L-nitroarginine methyl ester (L-NAME). Significant increase ($^{*}p<0.01$) in serum creatinine (Cr) occurred 24 h after injection of 8 ml kg^{-1} of diatrizoate. Serum Cr returned to a level comparable with baseline (pre insult) 7 days after the injection of diatrizoate($n=5$).

Experiment 4

The calcium antagonist diltiazem (10 mg kg^{-1}) offered significant protection against the reduction in renal function induced by 6 ml kg^{-1} of diatrizoate (Figure 4). Rats pre-treated with diltiazem (10 mg kg^{-1}) showed no significant rise in serum Cr (serum Cr at baseline 70.31±7.28 μmol l^{-1} and 78.27±17.85 μmol l^{-1} 24 h post **contrast**) but there was some reduction in Cr clearance (percentage decrease in Cr clearance 17.3±4.7%, $p<0.01$). This reduction was significantly less in comparison with the other groups (Figure 4b). Some protection was also observed in rats pre-treated with lower doses of diltiazem, but a significant serum Cr increase and Cr clearance reduction remained (Figure 4).

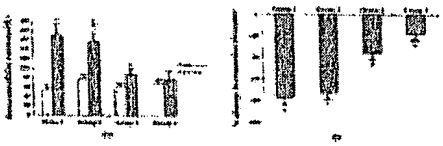

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Figure 4. The effect of the calcium antagonist diltiazem in preventing radiographic **contrast** medium induced-nephropathy in rats pre-treated with indomethacin and L-nitroarginine methyl ester (L-NAME). (a) Serum creatinine(Cr) before (□) and 24 h after (▨) injection of 6 ml diatrizoate in rats following intraperitoneal injection of either 4 ml of normal saline (Group 1) or diltiazem (Group 2, 2 mg kg^{-1}; Group 3, 6 mg kg^{-1}; or Group 4, 10 mg kg^{-1}). Significant increase ($^{*}p<0.01$) in serum Cr occurred in Groups 1, 2 and 3 but no significant rise

occurred in Group 4 (n=5 per group). Comparison of the percentage rise in serum Cr from baseline between groups was: Group 1>Group 2 ($p<0.05$), Group 2>Group 3 ($p<0.05$) and Group 3>Group 4 ($p<0.01$). (b) Percentage change in Cr clearance from baseline (24 h pre injection) to 24 h post injection of 6 ml diatrizoate in rats with introperitoneal injection of either 4 ml of normal saline (Group 1) or diltiazem (Group 2, 2 mg kg^{-1}; Group 3, 6 mg kg^{-1}; or Group 4, 10 mg kg^{-1}). Significant decreases ($^{*}p<0.01$) in Cr clearance occurred in all groups (n=5 per group). The smallest reduction occurred in Group 4. The extent of reduction was: Group 2>Group 3 ($p<0.01$), Group 3>Group 4 ($p<0.01$). No significant difference was found between Groups 1 and 2.

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Animal models are required for the study of the pathophysiology of RCMN. It is important that *in vivo* animal models are simple but clinically relevant. RCMN in man often occurs in patients with endothelial dysfunction, such as those suffering from diabetes mellitus, hypertension and atherosclerosis. It is also well documented in the literature that the intrarenal vasodilators prostacycline and nitric oxide, which are produced by healthy endothelium, are important for the perfusion of the renal medulla, which is particularly vulnerable to the ischaemic insult associated with the intravascular administration of RCM [12]. Whilst normal rats are resistant to the development of RCMN, combined acute inhibition of the synthesis of nitric oxide and prostacycline via the administration of L-NAME and indomethacin, respectively, predisposes rats to severe renal injury from RCM [12]. Implementing these acute pharmacological manipulations to inhibit the renal synthesis of nitric oxide and prostacycline obviated the need for chronic preparation or surgical intervention required in other experimental animal models [8]. This study, in agreement with previous reports, has shown the reliability of this simple animal model in studying RCMN. It has reconfirmed that RCMN is dose dependent and the reduction in renal function can spontaneously recover within 7 days of RCM administration [2, 13, 14]. It has also shown the renal tolerance to a non-ionic low osmolar RCM to be higher in comparison with high osmolar ionic **media**. These findings are compatible with clinical observations, including the lower frequency of RCMN in patients with impaired renal function receiving a low osmolar RCM in comparison with those injected with high osmolar **media** [2, 14–16].

Calcium ions play a crucial role in the physiology of smooth muscle cells. Constriction of smooth muscle cells is a function of intracellular calcium ion concentration. Although the sarcoplasmic reticulum contains an intracellular calcium ion pool that can be mobilized to give transient increases in the myoplasmic calcium ion concentration, sustained contraction of smooth muscle is totally dependent on the extracellular calcium pool and its influx. Central to the efficacy of calcium channel blockers is their ability to reduce transmembrane movement of calcium ions through the voltage-sensitive, calcium ion-selective channels. They disrupt excitation–contraction coupling by specific binding to high affinity sites in the plasmalemma. Calcium channel blockers therefore exert a pronounced vasorelaxant effect in the kidney and in other vascular beds. In addition to their complex influence on the renal microvascular

circulation, calcium channel blockers have a cytoprotective effect on renal cells by a number of additional mechanisms. These include inhibition of intracellular calcium "overload" after ischaemic or toxic injuries, a decrease in free radical formation, the modulation of mesangial traffic of macromolecules, a reduction in renal hypertrophy and even the control of immune response [17].

The role of calcium channel blockers in preventing RCMN has been investigated in both experimental animal models and clinical trials. It has been shown in the dog that calcium channel blockers inhibit RCM-induced intrarenal vasoconstriction [18]. However, results of clinical trials have been conflicting and the dosage of calcium channel blockers used in these studies could be a responsible factor. Prospective randomized clinical studies have shown that 3-day pre-treatment with a calcium channel blocker (20 mg day^{-1} nitrendipine orally, starting 1 day before **contrast** medium injection) protected against RCMN [19]. On the other hand, a single dose of 10 mg or 20 mg nitrendipine orally 1 h prior to RCM injection failed to prevent the development of RCMN [20, 21]. Our experimental results suggest that calcium channel blockers can offer good protection against RCMN, but this effect is dose dependent. Small doses (<10 mg kg^{-1} body weight) of the calcium channel blocker diltiazem did not provide good protection against the reduction in renal function induced by RCM. Further clinical studies using appropriate dosage are required to confirm the effectiveness of this class of drugs in prevention of RCMN.

Effective prevention of RCMN remains a contentious subject. The use of a low osmolar RCM, adequate hydration and volume expansion with saline infusion for several hours before and after RCM injection offer some protection against this complication [14]. The use of adenosine receptor antagonists, such as theophylines, have also been advocated, but clinical experience remains limited [2]. Endothelin (ET) antagonists have also been considered, but the only single clinical study using ET non-selective receptor antagonists has shown that this class of drug does not offer any protection [22]. However, the use of a selective ET-A receptor antagonist may offer some protection [4]. It is of interest that ET-induced vasoconstriction can also be prevented by calcium channel blockade [23]. It is therefore reasonable to suggest that a calcium channel blocker may have the same effect as an ET-A receptor antagonist in protecting the kidney against the ischaemic insult of RCM. Further investigations are required to compare the effectiveness of these two classes of drug in preventing RCMN.

A recent study has shown that RCMN can be prevented by the prophylactic administration of the antioxidant acetylcystein (600 mg orally twice daily, 24 h before and continued for 24 h after RCM injection) and hydration with 0.45% saline (1 mg kg^{-1} body weight) infused 12 h before and 12 h after **contrast** injection [24]. However, the number of patients recruited in this study was small and small doses of iv RCM (75 ml of 300 mgI ml^{-1} iopromide) were given to patients. 12% of these patients had an increase in serum Cr level of more than 44 μmol l^{-1} within 48 h after **contrast** medium administration; 2% in the acetylcysteine group and 21% in the control group. Further studies are required to validate the effectiveness of acetylcystine in preventing RCMN, particularly when larger doses of RCM are used or following intra-arterial administration. Nevertheless, the low cost of acetylcystine, its general availability, its limited side effects and its ease of administration makes the drug very attractive for routine use in prevention of RCMN.

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1. Morcos SK, El Nahas AM. Advances in the understanding of the nephrotoxicity of radio**contrast media**. Nephron 1998;78:249–52.[Medline]
2. Morcos SK. **Contrast media** induced nephrotoxicity—questions and answers. Br J Radiol 1998;71:357–65.[Abstract/Free Full Text]
3. Deray G, Jacobs C. Radio**contrast** nephrotoxicity: a review. Invest Radiol 1995;30:221–5.[Medline]
4. Idee JM, Bonnemain B. Reliability of the experimental models of iodinated **contrast media**-induced acute renal failure. From methodological considerations to pathophysiology. Invest Radiol 1996;31:230–41.[Medline]
5. Oldroyd S, Morcos SK. Endothelin: what does the radiologist need to know? Br J Radiol 2000;73:1246–51.[Abstract/Free Full Text]
6. Oldroyd SD, Fang L, Haylor JL, Yates MS, El Nahas AM, Morcos SK. Effects of adenosine receptor antagonists on the responses to **contrast media** in the isolated rat kidney. Clin Sci 2000;98:303–11.[Medline]
7. Heyman SN, Rosen S, Brezis M. Radio**contrast** nephropathy: a paradigm for synergism between toxic and hypoxic insults in the kidney. Exp Nephrol 1994;2:153–7.[Medline]
8. Oldroyd SD, Haylor JL, Morcos SK. Bosentan, an orally active endothelin antagonist: effect on renal response to **contrast media**. Radiology 1995;196:661–5.[Abstract]
9. Erley CM, Burgert K, Langanket J, Osswald H, Risler T. A novel animal model of radio**contrast**-induced nephropathy (RCN). J Am Soc Nephrol 1995;5:426.
10. Margulies KB, Mckinley LJ, Cavero PG, Burnett JC. Induction and prevention of radio**contrast**-induced nephropathy in dogs with heart failure. Kidney Int 1990;38:1101–8.[Medline]
11. Duarte CG, Zhang J, Ellis S. The SHR as a small animal model for radio**contrast** renal failure. Relation of nephrotoxicity to animal's age, gender, strain, and the dose of radio**contrast**. Ren Fail 1997;19:723–43.[Medline]
12. Agmon Y, Peleg H, Greenfield Z, Rosen S, Brezis M. Nitric oxide and prostanoids protect the renal outer medulla from radio**contrast** toxicity in the rat. J Clin Invest 1994;94:1069–75.[Medline]
13. Rudnick MR, Berns JS, Cohen RM, Goldfarb S. Nephrotoxic risks of renal angiography: **contrast media**-associated nephrotoxicity and atheroembolism. A critical review. Am J Kidney Dis 1994;24:713–27.[Medline]
14. Morcos SK, Thomsen HS. Webb JAW and the members of the **Contrast Media** Safety Committee of the European Society of Urogenital Radiology. **Contrast media** induced nephrotoxicity: a consensus report. Eur Radiol 1999;9:1602–13.[Medline]
15. Barrett BJ, Carlisle EJ. Mataanalysis of the relative nephrotoxicity of high- and low-osmolality iodinated **contrast media**. Radiology 1993;188:171–8.[Abstract]
16. Rudnick MR, Goldfarb S, Wexler L, et al. For the iohexol cooperative study. Nephrotoxicity of ionic and nonionic **contrast media** in 1196 patients: a randomized trial. Kidney Int 1995;47:254–61.[Medline]
17. Bakris GL, Burnett JC. A role for calcium in radio**contrast**-induced-reductions in renal hemodynamics. Kidney Int 1985;27:465–8.[Medline]
18. Neumayer HH, Junge W, Kufner A, Wenning A. Prevention of radio**contrast-media**-induced nephrotoxicity by the calcium channel blocker nitrendipine: a prospective randomized clinical trial. Nephrol Dial Transplant 1989;4:1030–6.[Abstract]
19. Khoury Z, Schlicht JR, Como J, Karschner JK, Shapiro AP, Mook WJ, et al. The effect of prophylactic nifedipine on renal function in patients administered **contrast media**. Pharmacotherapy 1995;15:59–65.[Medline]
20. Carraro M, Mancini W, Artero M, Stacul F, Grotto M, Cova M, et al. Dose effect of nitrendipine on urinary enzymes and microproteins following non-ionic radio**contrast** administration. Nephrol Dial Transplant 1996;11:444–8.[Abstract]

21. Neumayer HH, Gellert J, Luft FC. Calcium antagonists and renal protection. Ren Fail 1993;15:353–8.[Medline]
22. Wang A, Holcslaw T, Bashore TM, et al. Exacerbation of radiocontrast nephrotoxicity by endothelin receptor antagonism. Kidney Int 2000;57:1657–80.
23. Kiowski W, Luscher TF, Linder L, Buhler FR. Endothelin-1-induced vasoconstriction in humans. Reversal by calcium channel blockade but not by nitrovasodilators or endothelium-derived relaxing factor. Circulation 1991;83:469–75.[Abstract]
24. Tepel M, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. N Engl J Med 2000;343:180–4.[Abstract/Free Full Text]

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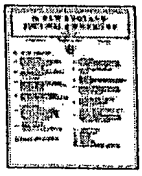

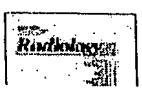

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Full paper

Changes in endothelial, leucocyte and platelet markers following contrast medium injection during angiography in patients with peripheral artery disease

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Abstract

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Peripheral artery angiography, a common diagnostic procedure, may cause early and late adverse reactions, such as anaphylaxis, thrombosis and possible progression of the underlying arterial disease. To test the hypothesis that radiographic **contrast** medium may contribute to these events by adversely affecting the endothelium, leucocytes and/or platelets, 19 subjects undergoing angiography for the investigation and/or treatment of lower limb atherosclerosis were recruited. Blood was obtained from the external iliac vein before, and at serial intervals after, the injection of radiographic **contrast** medium into the ipsilateral femoral artery for diagnostic use. Markers of endothelial cell injury (von Willebrand factor (vWf)), platelet activation (soluble P-selectin) and leucocyte activation (neutrophil elastase and soluble L-selectin) were measured in citrated plasma. Soluble intercellular adhesion molecule-1 (sICAM-1) and thromboxane B_2, which are non-specific markers of inflammation, were also measured. Compared with the sample prior to angiography, levels of soluble L-selectin and sICAM-1 were reduced ($p<0.02$) immediately after passage of the last bolus of **contrast** medium. 15 min later, levels returned to normal but the level of vWf had increased ($p<0.02$). After 30 min, only levels of thromboxane B_2 were increased ($p<0.05$). The following day both vWf ($p<0.01$) and soluble P-selectin ($p<0.05$) were increased. These data point to both early and late effects of **contrast** medium on markers of endothelial, platelet and leucocyte function.

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Diagnostic angiography is an essential investigation in the planning of surgical or interventional radiology treatment for abdominal and lower limb atherosclerotic disease. The use of intravascular **contrast media** may be associated with unfavourable reactions, such as a feeling of mild warmth or discomfort in the lower limbs, although serious adverse reactions such as anaphylactic shock are very rare. Various alterations in the functional properties of different blood cells and endothelial cells, such as in coagulation, have been reported [1, 2]. Some of these reactions may be owing to adverse or cytotoxic effects of radiographic **contrast** medium, the catheterization process, the X-rays themselves, or any combination of these on the endothelium, coagulation system, leucocytes and platelets [3]. Evidence for this hypothesis comes from *in vitro* experiments in which blood and **contrast media** are simply mixed and the properties of the resultant complex analysed [4–7], studies with cultured human and bovine endothelial cells [8–10], and animal experiments [11–16].

There have also been reports of the effects of **contrast** medium on humans *in vivo*. These include evidence of increased thrombotic and coagulation activity (raised thrombin–antithrombin III complexes, prothombin fragments 1+2 and D-dimers [6, 17]), reduced levels of alpha-2-antiplasmin [6], the consumption of complement components and a neutrophil leucocytosis [18], and increased tissue plasminogen activator (arising from platelets and endothelial cells) and beta-thromboglobulin (a platelet-specific marker) [19]. Studies of endothelium, platelets and neutrophil leucocytes (cells fundamental to the pathogenesis of atherosclerosis) *in vivo* are limited in this respect.

We hypothesized that there would be both early (within 30 min) and late (overnight) effects of **contrast** medium on endothelial cell, platelet and leucocyte function. To test this we measured levels of certain specific, relevant, recognized and well characterized markers of these cells. These were endothelial product von Willebrand factor (vWf), platelet product soluble P-selectin (sP-selectin) and leucocyte markers soluble L-selectin (sL-selectin) and neutrophil elastase. We also measured levels of soluble intercellular adhesion molecule-1 (sICAM-1) arising from the endothelium, activated lymphocytes and other cells, and thromboxane B_2 released from the endothelium, neutrophils and platelets.

Increased plasma levels of vWf found in cancer, atherosclerosis and connective tissue disease reflect endothelial cell damage/injury and are associated with the development of adverse events such as myocardial infarction and stroke. However, it is unclear whether this added risk is a direct consequence of endothelial dysfunction and/or an increased risk of thrombosis, which vWf can promote by cross-linking platelets [20]. Increased levels of sP-selectin are becoming recognized as reflecting platelet activation, and therefore thrombotic potential, *in vivo* [21, 22] and, like vWf, are present in atherosclerosis and cancer. They may also carry an increased risk of cardiovascular events [23, 24]. The membrane-bound form of L-selectin, found on leucocytes, **media**tes binding of these cells to the endothelium. Although sL-selectin in the plasma retains biological activity and inhibits this adhesion *in vitro*, other functions are yet to be clarified [25, 26]. sICAM-1 and thromboxane B_2, both non-specific markers of inflammation and both raised in the plasma of patients with peripheral artery disease [27, 28], were included to determine a possible inflammatory effect of the **contrast** medium, as atherosclerosis is believed to have an inflammatory component [29]. Our experimental approach was broadly similar to

those of other workers [6, 17–19], *i.e.* taking serial plasma samples before and after the injection of **contrast** medium in the diagnosis and treatment of atherosclerosis.

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This project was approved by the Ethics Committee of South Manchester Health Authority. Informed, written consent was obtained from all participants. The project conformed to the guidelines of the Declaration of Helsinki [30].

Subjects

19 patients were recruited from among those scheduled to undergo diagnostic radiography and/or angioplasty to investigate and/or treat presumed lower limb peripheral artery disease. Clinical and demographic details of the subjects are presented in Table 1. 45% of the interventions were performed on the left side. 70% of subjects were men and the mean±SD age of all subjects was 63±12 years. 24 age- (mean±SD, 59±9 years) and sex-matched (67% men) healthy control subjects were drawn from attenders for endoscopy, hernia repair or minor operations, and from healthy hospital staff. None of the control subjects displayed symptoms of vascular disease or signs, *e.g.* carotid bruit, on clinical examination. Exclusion criteria for all subjects were venous ulceration, serological evidence of hepatitis B virus or HIV infection, malignancy, acute or chronic liver and kidney disease, connective tissue disease, as well as the treatment or use of aspirin, thienopyridines, gpIIb/IIIa blockers, Warfarin, vasopression, or immunosuppressive or cytotoxic drugs. Systolic and diastolic blood pressure was recorded in each subject following a minimum of 5 min rest. Subjects were asked if they regularly smoked cigarettes, were ex-smokers or if they had never smoked.

View this table: Table 1. Clinical, angiographic and demographic details of the 19 patients
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Protocol

Following local anaesthesia using lignocaine, a 5 F catheter was inserted into the distal external iliac vein on the side that the investigation was to be performed. A blood sample (sample 1) was taken. The ipsilateral femoral artery was then punctured under local anaesthesia and the aorta was catheterized with a 4 F straight catheter with eight side holes. Diagnostic catheters and guidewires were flushed with heparinized saline. Up to 11 20 ml boluses of the **contrast** medium iohexol (Omnipaque 350, containing 350 mg iodine ml^{-1} (Nycomed Amersham plc, Chesham, UK)) diluted with heparinized saline (2000 U l^{-1}) to 240 mgI ml^{-1} were then injected by an automated pressure syringe (Medrad) at 6 ml s^{-1}. All procedures were performed by a single radiologist (R Ashleigh). Eight patients had angioplasty with an appropriately sized 5 F balloon catheter following the diagnostic angiogram, with a **median** of four (range two to eight) inflations. Thrombolytic agents were not used. A second blood sample (sample 2) was obtained after injection of the final bolus of **contrast** medium. Additional blood samples were taken from the venous line at 15 min (sample 3) and 30 min (sample 4) after last passage of the **contrast** medium. The femoral vein catheter was then removed and the patient returned to the ward. A final sample (sample 5) was taken from an antecubital vein the following morning on the ward.

Blood processing

Blood was collected into EDTA, sodium citrate or no anticoagulant. Plasma or serum was obtained by centrifugation at 2500*g* for 10 min at 4 °C and then frozen at -70 °C to allow batch analysis. vWf and sP-selectin were measured by a commercial ELISA (Dako, Denmark, and Takara Shuzo, Shiga, Japan, respectively) of the citrated sample. sL-selectin and sICAM-1 (R&D Systems, Abingdon, UK) were measured in the serum sample. Thromboxane B_2 was measured in citrated plasma by an ELISA from Cascade Biochemicals (Reading, Berkshire, UK). Fibrinogen in the initial and final citrated plasma samples wasmeasured by the Clauss technique with thrombin (Baxter, Deerfield, IL). Plasma elastase was measured in EDTA-plasma by an ELISA using sheep anti-human elastase and peroxidase-conjugated sheep anti-human antitrypsin (The Binding Site, Birmingham, UK), and a polymorphonuclear leucocyte elastase calibrator (Merck Ltd, West Drayton, UK) [31]. Intra-assay and inter-assay variancesof all assays were <5% and <10%, respectively.

Data analysis and statistics

Raw data were analysed by the Ryan–Joiner normality test to determine the nature of its distribution. sP-selectin, elastase and thromboxane B_2 were non-normally distributed and are therefore presented as **median** and range; all other data were normally distributed and so are presented as mean and standard deviation. Data between the cases and controls were compared with a *t*-test or the Mann–Whitney *U*-test. Fibrinogen data from samples 1 and 5 were analysed by paired *t*-test. Data from all samples were analysed by Friedman's repeated measures (two-way) analysis of variance, following log transformation where necessary.

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Table 2 and Figure 1 show levels of sICAM-1, vWf and sP-selectin in control subject and patient samples. Levels of sICAM-1 were reduced ($p<0.01$) immediately after angiography (sample 2). There was a transient rise in levels of vWf ($p<0.025$) after 15 min (sample 3). The following morning (sample 5), sICAM-1 was normal but levels of vWf ($p<0.01$) and sP-selectin ($p<0.05$) were increased (Figure 1). Levels of vWf ($p=0.031$), sICAM-1 ($p=0.028$) and sP-selectin ($p=0.042$) were all slightly higher in the patients' baseline blood samples compared with the controls' samples.

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Table 2. Levels of soluble intercellular adhesion molecule-1 (sICAM-1), von Willebrand factor (vWf) and soluble P-selectin (sP-selectin) levels in the 19 patients and 24 control subjects

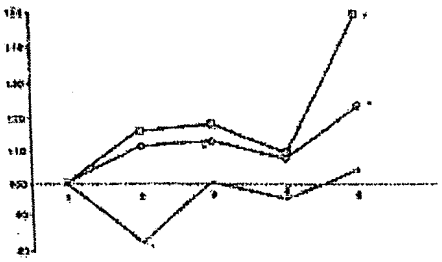

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Figure 1. Changes in levels of soluble intercellular adhesion molecule-1 (•) von Willebrand factor (○) and soluble P-selectin (□) in blood samples 1–5, expressed in terms of baseline levels at the start of the procedure. *$p<0.05$.

Table 3 and Figure 2 show levels of sL-selectin, elastase and thromboxane B_2 in control subject and patient samples. Levels of sL-selectin were reduced in samples 2 ($p<0.05$) and 4 ($p<0.05$). There were no significant changes in levels of elastase, but levels of thromboxane B_2 were increased at 30 min post **contrast** (sample 4) ($p<0.05$). None of the levels were significantly altered in the sample taken the following morning. There was no significant difference in levels of sL-selectin ($p=0.652$) or elastase ($p=0.210$) between patients and controls, but thromboxane B_2 was slightly higher in the patients' samples ($p=0.041$).

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Table 3. Levels of soluble L-selectin (sL-selectin), thromboxane B_2 and elastase levels in the 19 patients and 24 control subjects

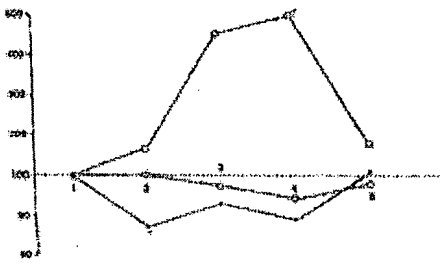

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Figure 2. Changes in levels of soluble L-selectin (•), thromboxane B_2 (□), and elastase (○) in blood samples 1–5, expressed in terms of baseline levels at the start of the procedure. *$p<0.05$.

Plasma fibrinogen was not significantly different in patients' sample 5 (3.8±1.2 g l^{-1}) compared with sample 1 (3.5±1.1 g l^{-1}, $p=0.21$), but was higher compared with the control subjects' levels (3.0±0.6 g l^{-1}, $p=0.022$). None of the clinical, angiographic or demographic factors described in Table 1, or age or sex, had an influence on any of the plasma markers. For example, the amount of **contrast** medium used or the number of balloon inflations or their duration all failed to correlate with the absolute levels of the six study indices or their relative increase or decrease from the initial sample. This is not surprising in view of the small number of patients. The small numbers also prevent meaningful subanalyses, such as angiography alone compared with angiography and angioplasty.

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The injection of radiographic **contrast** medium is associated with a variety of adverse events [1, 2, 32, 33]. We have found early (approx. 30 min) and late (approx. 24 h) changes in markers of endothelial cell and platelet function *in vivo*. Although all subjects were exposed to localized short bursts of ionizing radiation, it is generally considered that changes are mainly owing to the **contrast** medium [1–19]. Levels of sL-selectin (derived from leucocytes [26]) and sICAM-1 (derived from many cells, including the endothelium and activated leucocytes [27]) both fell im**media**tely after injection of the **contrast** medium bolus. Soluble adhesion molecules retain functional activity [26] and may be important in modulating adhesion of blood cells to the endothelium. The fall in sL-selectin and sICAM-1 levels seen here could reflect increased binding, and thus sequestration from the plasma, owing to activation-induced expression of ligands such as CD11a/CD18 on leucocytes and/or the endothelium [34]. Theoretically, any dilution effects of the **contrast** medium may contribute to this 13–18% reduction in sL-selectin and sICAM-1. However, this alone is unlikely to be the sole cause as, for example, elastase fell by only 5% and levels of vWf and thromboxane B_2 both increased.

Raised levels of vWf were present 15 min after **contrast** medium injection, suggesting an acute endothelial response. The high levels of vWf and sP-selectin after 24 h are probably owing to a late effect on the endothelium and platelets, respectively, complementing the report that **contrast media** induce increased expression of membrane P-selectin by platelets *in vitro* [35]. No change in levels of fibrinogen at this time implies the lack of an acute phase response so, as vWf may behave as an acute phase reactant [36], the increased levels are unlikely to be owing to a non-specific inflammatory increase. However, Tschopl et al [17] noted raised plasma vWf and fibrinogen after 48 h, but no increase after 1 h or 24 h, interpreting the fibrinogen increase as an acute phase response or as owing to increased synthesis by the liver, and the rise in vWf as increased synthesis by endothelial cells. Levels of the inflammatory **media**tor and vasoconstrictor thromboxane B_2, possibly deriving from the endothelium, platelets and neutrophils [28], rose markedly during the procedure, reaching significance after 30 min. Thromboxane B_2 has several effects, such as regulating vascular tone and resistance, although increased plasma levels may not necessarily indicate changes in the function of abluminal **medial** smooth muscle cells.

Our protocol is similar to that used by others [6, 17–19, 37]. Polanowska et al [19] investigated 26 men with peripheral artery disease and found a 20% increase in platelet marker beta thromboglobulin and a 32% increase in tissue plasminogen activator. They concluded that angiography may be responsible for partial stimulation or damage both of platelets and endothelial cells. We found no im**media**te effect on our platelet marker sP-selectin, although changes in other platelet markers *ex vivo* have been reported [38]. Hoffman et al [37] reported changes in several haemostatic markers 5 min and 30 min after exposure to **contrast** medium. Unlike us, they found no increase in vWf after 30 min but did find an increase in a different platelet marker (beta thromboglobulin) after 30 min. However, both our own study and that of Hoffman et al failed to find differences in leucocyte elastase, and so are to some extent complementary. Others have reported a reversible fall [39], or no acute change [40] in levels of beta thromboglobulin after the use of **contrast** medium, but Kolarov et al [41] reported platelet activation and consumption up to 2 h after the use of **contrast** medium.

One major difference between our own study and those outlined above is that we have attempted to localize any influence of **contrast** medium by obtaining our samples from the draining venous circulation of the limb most likely to be affected. In this way we hoped to avoid any haemodilution likely to occur when using a cubital vein. Consequently, our samples up to 30 min seem likely to be more sensitive to the effects of the **contrast** medium. The overnight sample carries with it the haemodilution issue so that we cannot say if the changes in levels of vWf and sP-selectin are due to systemic or local effects on the endothelium and platelets. A further deficiency in our study is the lack of a control group, although it would be impossible to subject patients to catheterization without the use of **contrast** medium or to use **contrast** medium without imaging. We are also unable to answer the point that our results may be owing only to the catheters and/or X-rays and not the **contrast** medium as we assume. However, we consider this unlikely in light of other *in vitro* data. Our single control group is of healthy, age- and sex-matched subjects that simply demonstrate what levels of markers would be expected in the absence of atherosclerosis—no direct case-control study is implied by these data. A further caveat is the possibility that the changes observed are simply owing to the effect of venepuncture/arteriopuncture alone and not necessarily the effect of the **contrast** medium. Cousins et al [42] found that arterial puncture, not the use of **contrast** medium, caused an increase in coagulation parameter fibrinopeptide A.

We believe this *in vivo* method provides the opportunity to extend *in vitro* and *in vivo* testing of the effects of different types of **contrast media** [3–7, 17, 37, 39–41]. At least some of the effects of Omnipaque 350 may be detected after 24 h, providing a convenient window for samples from an appropriate venous site. Indeed, our observations of adverse effects on the endothelium and platelets (marked by raised vWf, thromboxane B_2 and sP-selectin levels) may be related to both immediate effects such as discomfort and long-term side effects such as thrombosis and restenosis, that could be attributable to **contrast media**. This therefore supports the recent view of Zhang et al [43], whose *in vitro* experiments suggest that **contrast media** induce a degree of endothelial cell injury and apoptosis and that these may be associated with side effects. With our clinical data, we cannot say exactly what type of adverse endothelial perturbation is present, be it damage, injury, activation, frank necrosis or apoptosis [43, 44]. We do not believe our data are simply reflecting an artefact of **contrast media** induced nephrotoxicity [10, 45] as the changes are clearly specific to certain molecules. If there was a degree of renal impairment, we may perhaps have expected similar changes in all the molecules, or a pattern, possibly related to their size. This was not the case as, for example, the soluble adhesion molecules are all of similar size (approximately 100–200 kDa), elastase is in the region of 27.5 kDa and thromboxane B_2 is 370 Da, yet all show different patterns.

The further clinical and cell biology implications of our findings are unclear. However, it may be that these adverse changes to the endothelium and platelets implying increased risk of coagulopathy and thrombosis, may also be related to the risks of restenosis that are common in patients undergoing arterial investigations [32, 33, 46, 47] and therefore warrant additional studies, especially in long-term follow-up. However, despite our focus on the endothelium and platelets, the cytotoxic effects of **contrast** medium on smooth muscle cells may also be important [48].

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Footnotes

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1. Ansell G. Complication of intravascular iodinated **contrast media**. In: Ansell G, Beltman MA, Kienfron JA, Wilkins RA, editors. Complications in diagnostic imaging and interventional radiology (3rd edn). Boston, MA: Blackwell Science, 1996:245–302.
2. Elroy R, Corot C, Belleville J. **Contrast media** for angiography: physicochemical properties, pharmacokinetics and biocompatibility. Clin Mater 1991;7:89–197.[Medline]
3. Fareed J, Moncada R, Messmore HL, Walenga JM, Hoppensteadt D, Wehrmacher WH. Molecular markers of **contrast-media** induced adverse reactions. Semin Thromb Hemost 1984;10:306–28.[Medline]
4. Englehart JA, Smith DC, Maloney MD, Westengard JC, Bull BS. A technique for estimating the probability of clots in blood/**contrast** agent mixtures. Invest Radiol 1988;23:923–7.[Medline]
5. Carr DH, Walker AC, White RG. Effects of radiographic **contrast media** on leukocyte locomotion. Invest Radiol 1981;16:133–40.[Medline]
6. Stormorken H, Skalpe IO, Testart MC. Effect of various **contrast media** on coagulation, fibrinolysis, and platelet function. An *in vitro* and *in vivo* study. Invest Radiol 1986;21:348–54.[Medline]
7. Corot C, Chronos N, Sabattier V. *In vitro* comparison of the effects of **contrast media** on coagulation and platelet activation. Blood Coagul Fibrinolysis 1996;7:602–8.[Medline]
8. Morgan DM, Bettmann MA. Effects of X-ray **contrast media** and radiation on human vascular endothelial cell *in vitro*. Cardiovasc Intervent Radiol 1989;12:154–60.[Medline]
9. Laerum F. Cytotoxic effects of six angiographic **contrast media** on human endothelium in culture. Acta Radiol 1987;28:99–105.[Medline]
10. Haller C, Schick CS, Zorn M, Kubler W. Cytotoxicity of radio**contrast** agents on polarised renal epithelial cell monolayers. Cardiovasc Res 1997;33:655–65.[Medline]
11. Heyman SN, Clark BA, Kaiser N, Spokes K, Rosen S, Brezis M, et al. Radio**contrast** agents induce endothelin release *in vivo* and *in vitro*. J Am Soc Nephrol 1992;3:58–65.[Abstract]
12. Riemann CD, Massey CV, McCarron DL, Borkowski P, Johnson PC, Ziskind AA. Ionic **contrast** agent-**media**ted endothelial injury causes increased platelet deposition to vascular surfaces. Am Heart J 1993;126:71–8.
13. Marguiles KB, Hildebrand FL, Heublein DM, Burnett JC. Radio-**contrast** increases plasma and urinary endothelin. J Am Soc Nephrol 1991;2:1041–5.[Abstract]
14. Fleetwood G, Bettmann MA, Gordon JL. The effects of radiographic **contrast media** on myocardial contractility and coronary resistance: osmolality, ionic concentration and viscosity. Invest Radiol 1990;25:254–60.[Medline]
15. Romano M, Di Bello M, Salmona M, Rosati G. Effect of iodinated **contrast media** on the synthesis and metabolism of leukotriene B4. Invest Radiol 1990;25:S25–6.[Medline]
16. Almen T, Aspelin P. Cardiovascular effects of ionic monomeric, ionic dimeric and non-ionic **contrast media**. Invest Radiol 1975;10:557–63.[Medline]
17. Tschopl M, Tsakiris DA, Marbet GA, Labs KH, Jager K. Role of hemostatic risk factors for

restenosis in peripheral arterial occlusive disease after transluminal angioplasty. Arterioscler Thromb Vasc Biol 1997;17:3208–14.[Abstract/Free Full Text]
18. Georgsen J, Rasmussen F, Antonsen S, Larsen MG. Influence of radiographic **contrast media** on granulocyte enzymes and complement during uncomplicated urographies. Eur J Radiol 1991;12:63–6.[Medline]
19. Polanowska R, Wilczynska M, Stawinski W, Goch JH, Augustyniak W, Cierniewski CS. Changes in platelet activity and tissue plasminogen activator during arteriography in patients with chronic limb ischaemia. Thromb Res 1992;65:663–5.[Medline]
20. Blann AD. von Willebrand factor and the endothelium in vascular disease. Br J Biomed Sci 1993;50:125–34.[Medline]
21. Blann AD, Lip GYH. Hypothesis: is soluble P-selectin a new marker of platelet activation? Atherosclerosis 1997;128:135–8.[Medline]
22. Fijnheer R, Frijns CJM, Korteweg J, Rommes H, Peters JH, Sixma JJ, et al. The origin of P-selectin as a circulating plasma protein. Thromb Haemost 1997;77:1081–5.[Medline]
23. Blann AD, Wadley M, Stonelake P, Gurney D, Bareford D, Lip GYH. Soluble P-selectin in patients with haematological and solid cancers. Blood Coagul Fibrinolysis 2001;12:9–16.[Medline]
24. Ridker PM, Buring JE, Rifai N. Soluble P-selectin and the risk of future cardiovascular events. Circulation 2001;103:491–5.[Abstract/Free Full Text]
25. Kansas GS. Selectin and their ligands: current concepts and controversies. Blood 1996;88:3259–87.[Free Full Text]
26. Schieffenbaum B, Spertini O, Tedder TF. Soluble L-selectin is present in human plasma at high levels and retains functional activity. J Cell Biol 1992;11:229–38.
27. van de Stolpe A, van der Saag PT. Intercellular adhesion molecule-1. J Mol Med 1996;74:13–33.[Medline]
28. Patrono C, Patrignani P, Davi G. Thromboxane biosynthesis and metabolism in cardiovascular and renal disease. J Lipid **Mediat** 1993;6:411–5.[Medline]
29. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.[Free Full Text]
30. World Medical Association Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. Cardiovasc Res 1997;35:2–3.[Medline]
31. Browser MS, Harpel PC. Alpha-1-antitrypsin-human leukocyte elastase complexes in blood quantified by an ELISA and comparison with alpha-2-plasmin inhibitor-plasmin complexes. Blood 1983;61:842–9.[Abstract]
32. Steinberg EP, Moore RD, Powe NR, Gopalan R, Davidoff AJ, Litt M, et al. Safety and cost effectiveness of high-osmolality as compared with low osmolality **contrast** material in patients undergoing cardiac angiography. N Engl J Med 1992;326:425–30.[Abstract]
33. Barrett BJ, Parfrey PS, Vavasour HM, O'Dea F, Kent G, Stone E. A comparison of non-ionic, low osmolality radio**contrast** agents with ionic high osmolality agents during cardiac catheterisation. N Engl J Med 1992;326:431–6.[Abstract]
34. Donnelly SC, Haslet C, Dransfield I, Robertson CE, Carter DC, Ross JA, et al. Role of selectins in development of adult respiratory distress syndrome. Lancet 1994;344:215–9.[Medline]
35. Chronos NAF, Goodall AH, Wilson DJ, Sigwart U, Buller NP. Profound platelet degranulation is an important side-effect of some types of **contrast media** used in interventional cardiology. Circulation 1993;88:2035–44.[Abstract]
36. Pottinger BE, Read RC, Paleolog EM, Higgins PG, Pearson JD. von Willebrand factor is an acute phase reactant in man. Thromb Res 1989;53:387–94.[Medline]
37. Hoffmann JJML, Tielbeek AV, Krause W. Haemostatic effects of low osmolar non-ionic and ionic **contrast media**: a double blind comparative study. Br J Radiol 2000;73:248–55.[Abstract/Free Full Text]
38. Grabowski EF, Jang IK, Gold H, Palacios IF, Boor SE, Rodino LJ, et al. Platelet degranulation induced by some **contrast media** is independent of their non-ionic *vs* ionic nature. Acta Radiol 1995;36:182–4.[Medline]
39. Brzosko M, Cyrylowski L, Brzosko I, Domanski Z, Fiedorowicz–Fabrycy I. Effects of ionic and non-ionic **contrast** medium on platelet function as evaluated by plasma concentrations of beta-

thromboglobulin. Br J Radiol 1997;70:1239–44.[Abstract/Free Full Text]
40. Vaitkus PT, Watkins MW, Witmer WT, Tracey RP, Sobel BE. Characterization of platelet activation and thrombin generation accompanying percutaneous transluminal coronary angioplasty. Coron Artery Dis 1995;6:587–92.[Medline]
41. Kolarov P, Tschoepe D, Nieuwenhuis HK, Gries FA, Strauer B, Schultheiss HP. PTCA: periprocedural platelet activation. Part II of the Duesseldorf PTCA platelet study (DPPS). Eur Heart J 1996;17:1216–22.[Abstract]
42. Cousins C, Dutka DP, Bradshaw A, Dawson P. Effect of arterial cannulation and **contrast** agents on blood coagulation. Acad Radiol 1995;2:663–6.[Medline]
43. Zhang H, Holt CM, Malik N, Shepherd L, Morcos SK. Effects of radiographic **contrast media** on proliferation and apoptosis of human vascular endothelial cells. Br J Radiol 2000;73:1034–41.[Abstract/Free Full Text]
44. Blann AD. Viewpoint: endothelial cell activation, injury, damage and dysfunction: separate entities or mutual terms? Blood Coagul Fibrinolysis 2000;11:623–30.[Medline]
45. Morcos SK. **Contrast media**-induced nephrotoxicity—questions and answers. Br J Radiol 1998;71:357–65.[Abstract/Free Full Text]
46. Von Andel GJ. Arterial occlusion following angiography. Br J Radiol 1980;53:747–53.[Abstract]
47. Spencer JA, Fletcher EWL. Deterioration following delay in performing femoral angioplasty. Br J Radiol 1990;63:919–21.[Abstract]
48. Wang YX, Chan P, Morcos SK. The effect of radiographic **contrast** medium on human vascular smooth muscle cells. Br J Radiol 1998;71:376–80.[Abstract/Free Full Text]

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A comparison between the platelet activating properties of different contrast media used in radiology and MRI

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Abstract. Previous studies have demonstrated that some intravascular radiographic contrast media (CM) used in angiography, especially non-ionic monomers, may cause platelet activation. This study was designed to elucidate which properties of the CM were responsible for this activity. Platelet activation engendered by CM was studied using flow cytometry to detect platelet degranulation (as CD62 expression) and fibrinogen binding. In order to elucidate the relevant characteristics of the CM responsible, contrast agents of differing structures, properties, formulations and osmolalities were studied; ionic and non-ionic, monomeric and dimeric. Gadolinium chelate MR enhancing agents and saline solutions of differing osmolalities were also investigated. Ionic dimeric sodium meglumine ioxaglate, non-ionic dimeric iodixanol and non-ionic dimeric iotrolan did not produce increased degranulation compared with saline controls. However, all agents produced a mild increase in bound fibrinogen. Experiments using saline solutions demonstrated that these effects are not attributable to the high osmolality of some CM. The broad comparison facilitated by this study shows that previous generalizations regarding platelet activation by CM, based on an ionic–non-ionic division, are not valid. We presume that some chemical structural property of the compounds is responsible and it is of note that the chemically distinct gadolinium chelates, gadolinium DTPA and gadolinium DTPA-BMA, also caused platelet activation to a similar degree. CD62 expression correlated with fibrinogen binding suggesting that at least one common pathway of platelet activation is involved.

Introduction

Since the observation by Robertson in 1987 [1] that blood has a greater tendency to form clots in angiographic syringes containing non-ionic rather than ionic contrast media (CM), considerable controversy has raged over their safety [2–20]. A number of different effects on several aspects of blood coagulation is thought to be responsible for this phenomenon but one important factor, only recently explored, is platelet activation [21, 22]. These studies have utilized a sensitive flow cytometry technique to detect platelet activation by using monoclonal antibodies to surface proteins. Degranulation occurs during platelet activation, and P-selectin (CD62), a component of alpha granule membranes, is exposed on the platelet surface. In addition the IIb–IIIa receptor undergoes a conformational change and becomes receptive to fibrinogen binding. Surface bound fibrinogen and surface CD62 can be detected using labelled antibodies and FACS analysis [23].

Several different non-ionic CM have been shown to cause platelet degranulation to varying degrees [21, 22]. The significance of this for the clinical use of these agents in angiography remains unclear but post-angiographic thrombosis and post-angioplasty restenosis are significant clinical problems and thromboembolic disasters during angiographic procedures are greatly feared [20]. The mechanisms by which CM produce such platelet activation are not defined. CM are conventionally classified according to their osmolality, ionic status and monomeric or dimeric structure. We have studied the effect of CM of each type on platelet activation *in vitro*, covering a wide range of osmolality and, for the first time, the new non-ionic dimeric agents iodixanol (Nycomed AS, Oslo) and iotrolan (Schering AG, Berlin). In an effort to determine the specificity of the degranulation effect we have also studied for the first time the effects of gadolinium chelate MR enhancing agents. We have further investigated the role of osmolality using saline solutions of differing concentrations. We conclude that there is no identifiable common physical characteristic that correlates with the effect on platelets and that some chemical property(s) of the media must be responsible.

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Materials and methods

Collection of blood samples

Blood was collected via a 19 gauge needle from healthy, non-smoking volunteers taking no medication. Care was taken to ensure there was no venous stasis and no excessive trauma on withdrawal of blood. The first 5 ml of blood was discarded and the subsequent 5 ml drawn directly into 5 ml of test solution (*i.e.* contrast medium or hepes buffered saline as control for sample manipulation) as previously described [21]. This is likely to reproduce the concentrations produced at the site of injection (*i.e.* 50:50, CM:blood).

After gentle mixing with test solution for 1 min, the sample was transferred to a vacutainer (Becton Dickinson) containing one-tenth volume trisodium citrate (0.015 M) as anticoagulant. The sample was then processed for flow cytometric analysis within 10 min.

Platelet activation, staining and cytometric analysis

Platelet activation was studied by measuring surface expression of CD62 and surface bound fibrinogen using immunocytometry, essentially as described by Janes et al [23] with minor modifications. The anti-CD62 monoclonal antibody, directly conjugated to FITC, was purchased from Harlan Sera-Lab Ltd (Sussex, UK). The negative control for this antibody was an IgG1 isotope purchased from the same source. An unconjugated anti-human fibrinogen polyclonal antibody (Harlan Sera-Lab) was used with an affinity isolated polyclonal rabbit anti-goat antibody conjugated to RPE (Sigma). The negative control consisted of affinity purified goat IgG (Harlan Sera-Lab) used with the RPE-conjugated detecting antibody. Whilst size and scatter characteristics permitted straightforward gating of the platelet population, positive staining for GpIb (CD42b) also confirmed platelet identity. The platelet specific antibody (anti-CD42b antibody directly conjugated to FITC) was purchased from Dako Ltd (Bucks, UK).

Samples were stained for platelet activation markers by adding 5 µl sample to a final reaction volume of 70 µl containing 5 µl of (1/5 diluted) anti-CD62-FITC antibody, 5 µl of (1/5 diluted) anti-human fibrinogen antibody, and 5 µl (1/20 diluted) anti-goat RPE antibody. All dilutions and reactions were performed in hepes buffered saline. For confirmation of platelet identity, the reaction volume contained 5 µl anti-CD42b antibody alone. In preliminary experiments to define activation, 5 µl thrombin was added to give final concentrations of 0.08 U ml^{-1} to 0.625 U ml^{-1}.

Samples were incubated for 20 min at 22–26 °C and the reaction stopped by the addition of 0.5 ml formyl saline. The fixed samples were analysed on a Becton Dickinson FACScan using Cell Quest® flow cytometry software. A minimum of 5000 gated events was analysed in each experiment.

Contrast media studied

The characteristics of the CM studied are set out in Table 1. They were chosen to represent a full range of physicochemical properties. In addition, two gadolinium chelates used for enhancement of MR were also included for comparison. The results obtained using the contrast media were compared in all cases with the saline control to eliminate effects due to the handling and manipulation of samples.

Results

Controls

Stimulation of whole blood with thrombin induced activation of platelets which could be analysed by flow cytometry (Figure 1). Over the concentration range 0.185 iu ml^{-1} to 0.625 iu ml^{-1} thrombin induced a gradual increase in CD62 expression and fibrinogen binding as expected (Figure 2). Control experiments demonstrated that when blood was collected directly into HBS,

Table 1. The different contrast media used in this study and their physicochemical characteristics

Name	Mono/dimer	Ionic/non-ionic	Osmolality	CD62	Fibrinogen
Hepes saline	—	—	290	—	—
Urografin (diatrizoate)	Mono	Ionic	2070	+	+ +
Omnipaque (iohexol)	Mono	Non-ionic	880	+ +	+ +
Hexabrix (ioxaglate)	Dimer	Ionic	580	ns	+
Visipaque (iodixanol)	Dimer	Non-ionic	290	ns	+
Isovist (iotrolan)	Dimer	Non-ionic	290	ns	+
Ultravist (iopramide)	Mono	Non-ionic	610	+ +	+ +
Optiray (ioversol)	Mono	Non-ionic	780	+ +	+ +
Magnevist (GdDTPA)	Gadolinium	Ionic	1960	+ +	+
Omniscan (GdDTPA-BMA)	Gadolinium	Non-ionic	650	+ +	ns

The last two columns summarise the effect of each on CD62 expression and fibrinogen binding of platelets.
+, $p<0.5$; + +, $p<0.001$; ns, not significant ($p>0.05$).

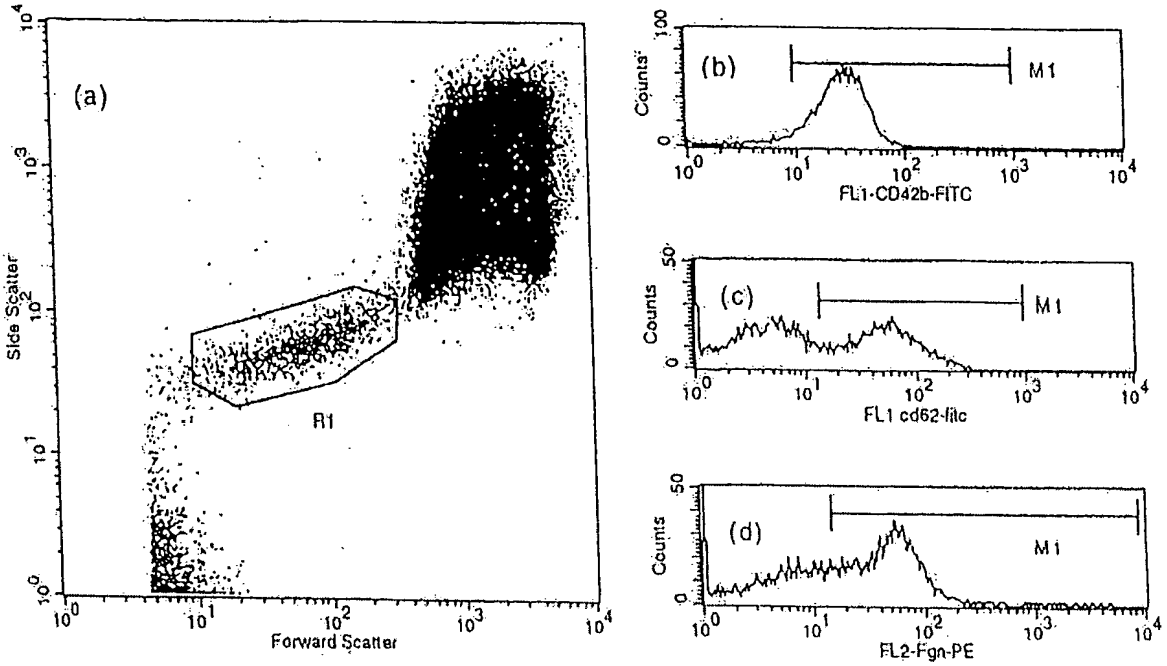

Figure 1. Flow cytometric analysis of whole blood. (a) Platelets, defined by their forward and side scatter characteristics, are shown in the gated region (R1). In this particular example blood was incubated with 0.273 U ml^{-1} thrombin, a sub-maximal stimulatory concentration which does not cause excessive platelet clumping or microvesiculation. Platelet identity of the events in R1 was confirmed by their expression of CD42b (b). In a separate analysis using an identical concentration of thrombin and gating region, levels of expression of CD62 (c), and binding of fibrinogen (d), are estimated. The analysis region M1 was set using isotype control antibodies.

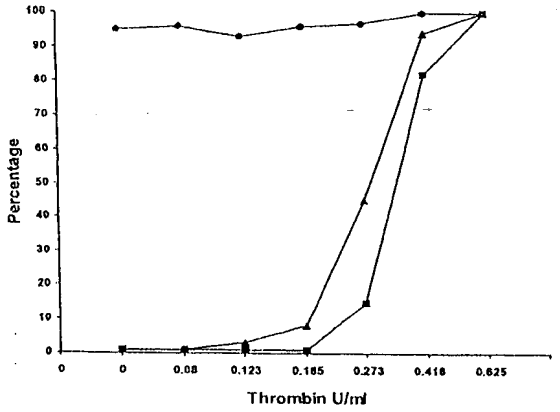

Figure 2. The effect of increasing thrombin concentration on CD62 expression (■) and fibrinogen binding (▲) by platelets (●) present in the gated analysis region (R1) shown in Figure 1. At a thrombin concentration of 0.625 U ml^{-1}, clumping of platelets and their subsequent removal from R1 prevented accurate estimation of activation. At this concentration, levels of activation markers are therefore assigned a value of 100%.

platelets showed very little surface expression of CD62 or binding of fibrinogen. At high concentrations of thrombin the platelets aggregated and were lost from the analysed gate. None of the CM studied produced this degree of activation or fibrinogen binding. Loss of platelets from the gated area (*i.e.* gated events as a proportion of total events) was not observed after mixing with CM.

CD62 expression

The most marked degree of CD62 expression was produced by the non-ionic monomers: Omnipaque (iohexol), Ultravist (iopromide) and Optiray (ioversol) (Figure 3). This is consistent with previous reports. However, the activation cannot be attributed to their non-ionic nature because Visipaque (iodixanol) and Isovist (iotrolan), which are also non-ionic, produced no significant degranulation. In addition, the ionic monomer Urografin (sodium meglumine diatrizoate) also produced significant degranulation. It is notable that none of the dimers, ionic (ioxaglate) or non-ionic (iodixanol, iotrolan), produced significant degranulation. This might at first glance be attributed to their generally lower osmolality (see below).

Fibrinogen

In contrast to previous reports we found that all but one of the agents produced a significant increase in surface binding of fibrinogen (Figure 4). Although this was generally small it was quite marked in the cases of Omnipaque (iohexol),

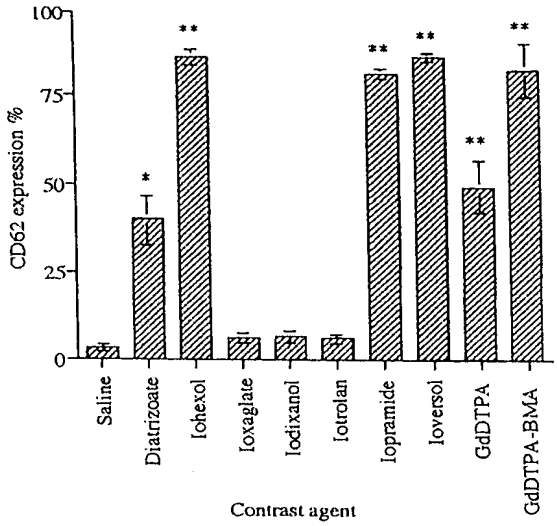

Figure 3. CD62 expression as % positive platelets after exposure to the CM shown. SEM of five experiments denoted by error bars. p is different from saline control by one-tailed t-test: * = $p<0.05$, ** = $p<0.001$.

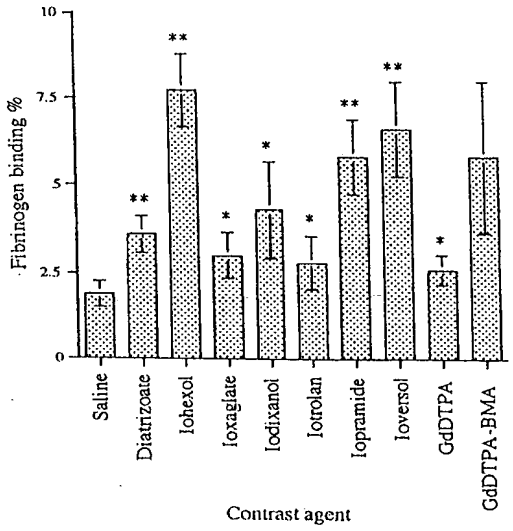

Figure 4. Surface bound fibrinogen shown as % positive platelets after exposure to the contrast agents as shown. SEM of five experiments denoted by error bars. p is different from saline control by one-tailed t-test: * = $p<0.05$, ** = $p<0.001$.

Ultravist (iopromide) and Optiray (ioversol) which had also marked effects on CD62 expression. Although the mean elevation of fibrinogen produced by Omniscan (GdDTPA-BMA) was apparently large, it did not reach significance due to the rather large interindividual variation in response. Such a marked degree of individual variation in response has been noted in previous studies [21]. Overall, there is a less marked variation between CM in the degree of induced fibrinogen binding than of CD62 expression.

Relationship of CD62 expression and fibrinogen binding

It is possible to activate platelets by a number of different pathways which have differential effects on degranulation and fibrinogen binding. The occasional discrepancy between the CD62 and the fibrinogen results raises the possibility that they arise via different mechanisms. Comparison of the two parameters is illustrated in Figure 5 and shows a good degree of correlation. The data are non-normalizable but by non-parametric Spearman ranking analysis show a significant correlation: $r=0.6$, $p=0.0001$. This suggests that these two markers of platelet activation are stimulated in part via a common, CM sensitive pathway but also that other factors are likely to be involved.

Osmolality

The low level of activation seen with the dimeric CM (ionic and non-ionic) suggested the effects of the other agents may be attributable to their generally higher osmolality. In order to investigate this possibility we performed experiments in a similar fashion but mixing blood instead with saline solutions of various higher osmolalities. The results are shown in Figure 6. Although the level of CD62 expression and fibrinogen binding appeared slightly higher in these solutions none of them were significantly different from normal saline controls. There is no effect observed with an increase in osmolality. There is therefore no appreciable effect of osmolality on platelet activation. Another clear indicator that osmolality is not the key factor is that GdDTPA-BMA has a greater effect than GdDTPA yet has a much lower osmolality.

Discussion

There is extensive literature documenting thrombotic and thromboembolic problems associated with angiography and, allegedly, with some CM [2–20]. More recently, the effect of CM on platelet activation and degranulation has been explored [21, 22]. Although much controversy exists over the relevance of *in vitro* findings to *in vivo* events, a survey of current practice suggests that many radiologists and cardiologists continue to take them seriously. Substantial numbers of radiologists and cardiologists use ionic CM (high or low osmolality) and heparin in various regimens in both diagnostic and interventional studies [24].

The accepted thromboembolic complication rate in the pre non-ionic era was ~0.2% [20, 25]. Comparable rates have been seen in large scale studies of non-ionic monomeric agents, even in the occasional paper calling attention to a supposed problem with the non-ionic agents [2].

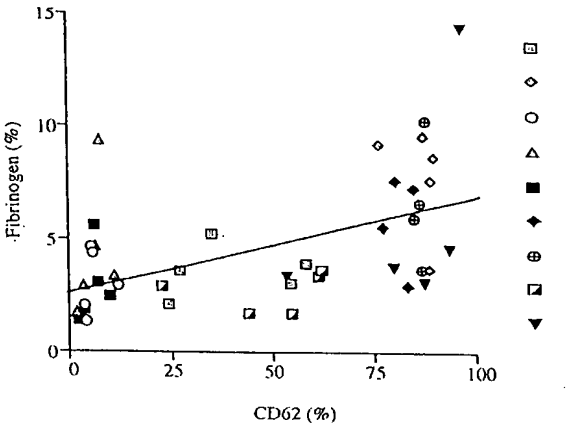

Figure 5. The relationship between CD62 expression and fibrinogen binding for each of the CM as shown with line of best fit. There is a significant correlation between these two measures of platelet activation ($r = 0.6$, $p = 0.0001$) by Spearman rank correlation test.

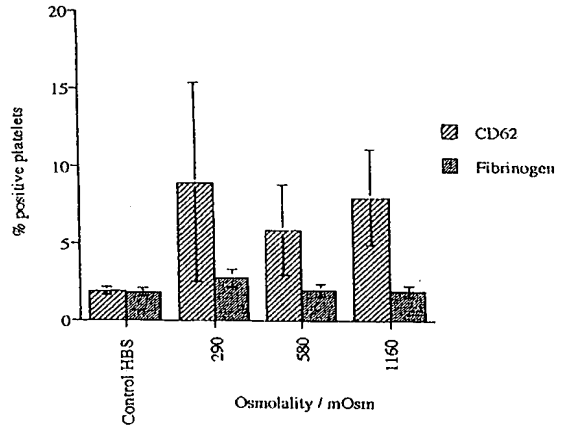

Figure 6. CD62 expression and fibrinogen binding of platelets after exposure to saline solutions of differing osmolalities as shown. The differences are not significant ($p > 0.05$ by one-tailed t-test).

Nevertheless, there have been persistent reports [10, 11] of a high incidence of such events with non-ionic agents, sufficient to keep levels of anxiety high, particularly among cardiologists. This has been the case despite the absence of controls and presence of confounding factors in some of these reports. Nonetheless, findings of this kind may be predicted from the effects of CM on platelet activation discussed here although such a conclusion rests on the extrapolation of *in vitro* results to *in vivo* clinical situations.

One difficulty in predicting or interpreting *in vivo* clinical results is that contrast agents have partly conflicting effects on the various parameters involved in thrombosis and thromboembolism. On the one hand they are all variably anticoagulant, largely by inhibiting fibrin polymerization; the non-ionic agents less so than the ionic [26, 27]. On the other hand, endothelial injury, a powerful mediator of platelet activation and coagulation activation, is much less marked than with the ionic agents [28]. Further, it is now demonstrated that at least some non-ionic agents also have pro-coagulant effects in engendering platelet activation. Previous and confusing results in the literature reporting apparent platelet inhibitory effects may be explained by the fact that profound platelet degranulation may result in circulating "exhausted platelets". These will respond poorly to conventional aggregation agonists *in vitro*.

Although platelets isolated systemically or even from the coronary sinus may not show signs of activation attributable to CM, the procedure itself may contribute to platelet activation [29]. The high concentrations of contrast present in the region of the catheter tip and adjacent to damaged endothelium may well be significant and will be comparable to the concentrations to which platelets are exposed in this study.

Several previous studies have reported the effect of different CM on platelet activation [21, 22]. Unfortunately, these have mixed and diverse methodologies. In this study we have used unmanipulated blood to expose platelets to CM by the most direct method and determined their effect on CD62 expression and on surface bound fibrinogen. In addition, previous studies have examined only a small number of different media with different physicochemical properties. This has tended to result in generalizations about the propensity of ionic or non-ionic CM to cause platelet activation. In this study a broad range of CM types has been used and it is clear that these generalizations are not valid. For example, Chronos et al [21] concluded that "any contrast media based on non-ionic molecules would be likely to cause platelet degranulation". The analysis of activation produced by iodixanol and iotrolan demonstrated in this study show that this is not in fact so. Some other property of the molecules must be responsible. Finally, this is the first study to include the gadolinium chelates used in MR which, while not relevant to clinical angiography, provide an interesting comparison as they are chemically quite distinct. This is also the first study of the new

non-ionic dimeric low osmolar CM, iodixanol and iotrolan which appear to have relatively good safety characteristics. The effect on platelets of Ultravist (iopromide) and Optiray (ioversol) have also not been previously reported.

In this study all three dimeric CM (ionic and non-ionic) failed to produce any significant degree of activation and no significant degranulation. The non-ionic dimeric agents are iso-osmolar with body fluids and the ionic dimeric agent (Hexabrix/sodium meglumine ioxaglate) has the lowest osmolality at any iodine concentration of all the other agents. It therefore seemed a plausible hypothesis that the higher osmolality of some CM may be responsible for their effect on platelets. We performed experiments to demonstrate that this is not so. Indeed a comparison of osmolality with degree of activation by the group as a whole supports this conclusion. However, a more complex contribution by osmolality cannot be excluded by the simplified system used in these experiments.

Overall, there appears to be a good correlation between the surface bound fibrinogen and the surface expression of CD62, suggesting that, broadly, they are measuring aspects of the same effect. However, it is interesting that GdDTPA-BMA produced marked platelet degranulation yet was the only agent not to cause a significant rise in platelet bound fibrinogen. Although this was perhaps attributable to a rather variable response to this agent it raises the possibility that stimulation via different pathways may be involved. In addition, the relationship between CD62 expression and fibrinogen binding produced by these agents is, in all cases clearly different from that produced by thrombin, suggesting that a thrombin independent pathway is involved. An alternative possibility is that the agents also have an inhibitory effect on fibrinogen binding to platelets but the correlation with CD62 expression, although weak, argues against the implication of interference by additional mechanisms. How these agents stimulate platelets to produce such effects remains undetermined.

We conclude that platelet activation and degranulation *in vitro* by contrast enhancing agents is variable from agent to agent and the underlying mechanisms remain unclear. Ionic *versus* non-ionic characteristics are not, contrary to previous reports, relevant. There is no evidence that osmolality is an important determining factor. The observation that gadolinium agents also cause this phenomenon is a surprising extension of this problem and may at some stage become clinically relevant. The low level of platelet activation produced by the non-ionic dimers is strongly in favour of their choice as CM for angiography but final proof of their safety can only come from clinical studies.

References

1. Robertson HJF. Blood clot formation in angiographic syringes containing non-ionic contrast media. Radiology 1987;163:621–2.
2. Grollman JH, King-Lui C, Astone RA, Lurie MD. Thromboembolic complications in coronary angiography associated with the use of non-ionic contrast medium. Cath Cardiovasc Diagn 1988;14:159–64.
3. Parvez Z, Moncada R, Fareed J, Messmore HL. Antiplatelet action of intravascular contrast media. Implications in diagnostic procedures. Invest Radiol 1984;19:208–11.
4. Ing JJ, Smith DC, Bull BS. Differing mechanisms of clotting inhibition by ionic and non-ionic contrast agents. Radiology 1989;172:345–8.
5. Egelhart JA, Smith DC, Maloney MD. A technique for estimating the probability of clots in blood/contrast agent mixtures. Invest Radiol 1988; 23:923–7.
6. Kopko PM, Smith DC, Bull BS. Thrombin generation in non-clottable mixtures of blood and non-ionic contrast agents. Radiology 1990;174:459–61.
7. Dawson P. Embolic problems in angiography. Semin Haematol 1991;28(Suppl 7):31–7.
8. Ip JH, Fuster V, Israel D, Badimon J, Chesebro JH. The role of platelets, thrombin and hyperplasia in restenosis after coronary angioplasty. J Am Coll Cardiol 1991;17(Suppl):77B–88B.
9. Hwang MH, Piao ZE, K mD, Giardina JJ. The potential risk of thrombosis during coronary angiography using non-ionic contrast media. Cath Cardiovasc Diagn 1989;16:209–13.
10. Esplugas E, Cequier A, Jara F, et al. Risk of thrombosis during coronary angioplasty with low osmolality contrast media. Am J Cardiol 1991;68:1020–4.
11. Gasperetti CM, Feldman MD, Burwell LR, et al. Influence of contrast media on thrombus formation during coronary angioplasty. J Am Coll Cardiol 1991;18:443–50.
12. Fareed J, Walenga JM, Saravia GE, Moncada R. Thrombogenic potential of non-ionic contrast media? Radiology 1990;174:321–5.
13. Granger CB, Gabriel DA, Reece NS, et al. Fibrin modification by ionic and non-ionic contrast media during cardiac catheterisation. J Am Coll Cardiol 1992;69:821–2.
14. Corot C, Perrin JM, Belleville J, Amiel M, Eloy R. Effect of iodinated contrast media on blood clotting. Invest Radiol 1989;24:390–3.
15. Raininki R, Biihelo M. Blood clot formation in angiographic catheters. Acta Radiol 1990;31:217–20.
16. Dawson P. Non-ionic contrast agents and coagulation. Invest Radiol 1988;S310–S317.
17. Grabowski EF. Effects of contrast media on erythrocyte and platelet interactions with endothelial cell monolayers exposed to flowing blood. Invest Radiol 1988;23:S351–S358.
18. Parvez Z, Moncada R. Nonionic contrast medium: effects on blood coagulation and complement activation *in vitro*. Angiology 1986;37:358–64.
19. Dawson P. Thrombogenic potential of non-ionic contrast media? Radiology 1990;177:280–5.
20. Dawson P. Thromboembolic complications in angiography. J Interven Radiol 1991;6:1–3.
21. Chronos NA, Goodall AH, Wilson DJ, Sigwart U, Buller NP. Profound platelet degranulation is an important side effect of some types of contrast media used in interventional cardiology. Circulation 1993;88:2035–44.

22. Hardeman MR, Konijnenberg A, Sturk A, Reekers JA. Activation of platelets by low-osmolar contrast media: differential effects of ionic and nonionic agents. Radiology 1994;192:563–6.
23. Janes SL, Wilson DJ, Chronos N, Goodall AH. Evaluation of whole blood flow cytometric detection of platelet bound fibrinogen on normal subjects and patients with activated platelets. Thromb Haemost 1993;70:659–66.
24. Jackson DM, Dawson P. Current usage of contrast agents, anticoagulant and antiplatelet drugs in angiography and angioplasty in the UK. Clin Radiol 1995;50:699–704.
25. Dawson P, Hemingway A. Contrast agent doses in interventional radiology. J Intervent Radiol 1987;2:145–6.
26. Dawson P, Hewitt P, Mackie IJ, Machin SJ, Amin S, Bradshaw A. Contrast, coagulation, and fibrinolysis. Invest Radiol 1986;21:248–52.
27. Stormorken H, Skalpe IO, Testart MC. Effects of various contrast media on coagulation, fibrinolysis and platelet function. Invest Radiol 1986;21:348–54.
28. Beynon HJ, Walport MJ, Dawson P. Contrast agents and endothelial injury. Invest Radiol 1994;29:195–7.
29. Gasperetti CM, Gonias SL, Gimple LW, Powers ER. Platelet activation during coronary angioplasty in humans. Circulation 1993;88:2728–34.

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Review article

Effects of radiographic contrast media on the lung

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Abstract

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The pulmonary adverse effects of intravascular use of water soluble radiographic contrast media (RCM) include bronchospasm, pulmonary oedema and increase in the pulmonary arterial blood pressure (Ppa). Symptomatic bronchospasm is rare but subclinical increase in airways resistance is common after intravascular injection of RCM. Experimental studies have demonstrated that the low osmolar ionic dimer ioxaglate can induce significant bronchospasm in comparison with other types of RCM. Histamine and endothelin, which are potent bronchoconstrictors and released in response to the administration of RCM, do not seem to mediate the bronchospastic effect of RCM. Pretreatment with corticosteroids or antihistamine does not appear to prevent RCM induced bronchospasm, but the administration of β_2 adrenergic agonist can abolish this adverse effect. RCM induced pulmonary oedema can be secondary to endothelial injury causing an increase in the permeability of the microcirculation. It may also occur in patients with incipient cardiac failure, when large doses of RCM particularly of the high osmolar type are used. A rise in Ppa induced by RCM seems to be secondary to an increase in pulmonary vascular resistance through direct effects on the pulmonary circulation. Low osmolar non ionic monomers induce the least changes in the pulmonary circulation and should be the contrast media of choice for intravascular use in patients with pulmonary hypertension. The mechanisms responsible for the effects of RCM on airway resistance and pulmonary circulation remain unclear. Intrabronchial administration of high osmolar water soluble RCM is dangerous and can induce severe bronchial irritation and pulmonary oedema. Low osmolar RCM are well tolerated by the lungs following aspiration with minimal histological reaction.

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The lung is an important target organ of the effects of water soluble radiographic contrast media (RCM). The pulmonary circulation is the first important vascular bed to receive RCM following intravenous injection and during the venous return after arteriographic examinations. There are several pulmonary adverse effects that may follow the intravascular injection of RCM, which include bronchospasm, pulmonary arterial hypertension and pulmonary oedema [1, 2]. In this review the effects of RCM on respiration and pulmonary circulation following intravascular administration will be discussed as well as the side effects associated with intrabronchial aspiiration of these agents.

Respiratory effects of RCM

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The effects of RCM on airways resistance, ventilation [respiratory rate, tidal volume] and blood gases will be discussed in this section.

Effects of RCM on airways resistance

The respiratory adverse reactions that have been reported with the intravascular use of RCM include apnea, dyspnea and bronchospasm [1–7]. Bronchospasm has been reported to be a contributory factor in 23% of moderate and 5% of severe adverse reactions to intravascular administration of RCM [1]. While symptomatic bronchospasm is rare, occurring in 0.01% of patients [1], subclinical bronchospasm as detected by a fall in forced expiratory volume in 1 s (FEV_1) is common and tends to be less pronounced with low osmolar non ionic RCM [3–6]. However, Wilson and Davies found that both high osmolar ionic and low osmolar non-ionic RCM produce a comparable fall in FEV_1 and forced vital capacity [7]. Experimental studies in the guinea-pig found that the high osmolar ionic monomer diatrizoate, the low osmolar non ionic monomer iopromide and the iso-osmolar non ionic dimer iotrolan did not induce a significant increase in airways resistance (R_{aw}) and only the low osmolar ionic dimer ioxaglate caused the most bronchospasm (Table 1) [8, 9]. Some clinical studies have also documented a higher incidence of allergic-like reactions with ioxaglate in comparison with other types of RCM [10–13]. The bronchospastic effect of the low osmolar ioxaglate can not be an osmotoxic effect and most likely due to its chemical structure [9]. The pathophysiology of the changes in airways resistance induced by RCM remains obscure and could be multifactorial. The underlying mechanism may involve the release of bronchospastic mediators such as histamine, endothelin (ET), 5-hydroxytryptamine, prostaglandins, thromboxane and bradykinin, cholinesterase inhibition, vagal reflex and a direct effect on the bronchi [5, 9, 14–19]. Contrast media can cause the release of histamine a potent bronchoconstrictor from mast cells and basophils both through a direct effect and indirectly by activating the complement system [14, 15]. *In vitro* studies have demonstrated dose-dependent histamine release from human lung mast cells (HLMC) and basophils in response to all types of RCM [14, 15]. The high osmolar diatrizoate induced the largest histamine release from human basophils and HLMC. Ioxaglate and iotrolan were effective

in inducing histamine release from human basophils but not from HLMC. The low osmolar non ionic monomer iopromide was relatively ineffective activator of histamine release from both HLMC and basophils (Table 1) [15]. The importance of histamine in mediating RCM induced bronchospasm has not been proven conclusively. Experimental studies have shown that pretreatment with anti-histamine H1 receptor antagonist did not prevent RCM induced increase in R_{aw} [8, 9]. Pretreatment with prednisolone also did not offer any protection against RCM induced bronchospasm in spite of using the two doses regimen recommended by Lasser et al [8, 9, 20–23]. The use of corticosteroid prophylaxis in preventing RCM reactions including bronchospasm is not widely endorsed. It has been suggested that the use of non-ionic agents alone is better in preventing all categories of reactions than the use of high osmolar ionic agents with corticosteroid prophylaxis [24, 25].

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Table 1. Summary of the different pulmonary effects of radiographic contrast media (RCM)

The role of ET in mediating the bronchospastic effects of RCM has also been investigated [9]. ET is a potent smooth muscle constrictor which, in the lung, produces an increase in vascular resistance and marked bronchospasm [9, 26]. Despite the use of a pharmacologically effective dose of non-selective ET antagonist, no protective effect was found against RCM induced bronchospasm in the guinea pig [9].

Leakage of fluids from the microcirculation into the lung tissues and bronchi may also cause an increase in airways resistance. Experimental studies in the guinea pig have not revealed fluid accumulation in the lungs and bronchi in association with RCM-induced rise in R_{aw} [9]. Furthermore, aerosolized $ß_2$ adrenergic agonist treatment was able to completely reverse RCM induced increases in R_{aw}, which suggests that any airway narrowing resulting from oedema is minimal [8, 9].

A role for cholinesterase inhibition or vagal reflex in mediating RCM induced bronchospasm has not been confirmed. A direct effect of RCM on bronchial smooth muscle cells is possible and contribution of other bronchospastic mediators such as leucotrienes and kinins requires further investigations.

Effects of RCM on ventialtion (respiratory rate and tidal volume) and blood gases

Optimum gaseous exchange across the alveoli and uptake by the capillaries requires a matching of pulmonary perfusion with alveolar ventilation (ventilation/perfusion ratio (V/Q ratio) maintenance) [27]. Adverse effects of RCM on pulmonary perfusion and/or ventilation could lead to changes in levels of arterial blood gases [27, 28]. Increasing the diffusion distance due to pulmonary oedema would also interfere with gaseous exchange [29, 30].

Detailed studies on the effects of RCM on ventilation are lacking. Experimental studies in the dog

demonstrate that RCM can induce an increase in breathing rate and tidal volume [28]. In the rat, RCM [high osmolar ionic monomer, low osmolar ionic dimer, low osmolar non ionic monomer and iso-osmolar non ionic dimer] caused a fall in PaO_2 associated with rise in $PaCO_2$. The high osmolar diatrizoate induced the greatest change [27]. The changes in the blood gases were thought to be most likely secondary to a decrease in alveoli blood flow since no bronchospsm, reduction in ventilation, or pulmonary oedema was found to explain the abnormal gases exchange [27]. The absence of RCM induced bronchospasm can be elucidated by the fact that rat airways are not as sensitive to chemical stimulus as those of the guinea-pig model that may behave in a way comparable with human airways [9].

The effects of RCM on pulmonary circulation will be discussed in details in the following section.

Effects of RCM on pulmonary circulation

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An increase in pulmonary artery pressure (Ppa) has been reported following the intravascular injection of RCM [31–44]. The sudden increase in Ppa is thought to contribute to the morbidity and mortality associated with pulmonary angiography particularly in patients with pulmonary hypertension [34–38]. There are conflicting reports in the literature about the mechanisms responsible for these effects [33, 34, 36, 39–44]. Some studies demonstrated that the rise in Ppa is secondary to an increase in pulmonary vascular resistance (PVR) [43, 44], while others indicated that it is due to an increase in cardiac output associated with a decrease in PVR [39–42]. The studies which suggested a fall in the vascular resistance the PVR was not directly measured and calculated from the formula PVR=(pulmonary artery pressure-pulmonary venous pressure)/cardiac output. The increase in cardiac output was attributed to reduced peripheral vascular resistance of the systemic circulation due to RCM induced vasodilatation [33, 34, 39–42]. The fall in PVR could be due to an increase in the capacity of the pulmonary vascular bed by recruitment of closed vessels and active vasodilatation of pulmonary arteries [44]. Experimental studies have shown that RCM can induce both dilatation and constriction of pulmonary arteries, but in systemic vascular beds they induce mainly vasodilatation except in the kidney where vasoconstriction predominates [45–47]. The studies, which showed an increase in PVR, suggested that this could be secondary to vasoconstriction, changes in the rheological properties of the red blood cells and/or pulmonary oedema [29, 30, 39, 45, 48, 49]. RCM induced pulmonary oedema is often secondary to endothelial injury leading to an increase in the permeability of the microcirculation and accumulation of fluid in the lung. Pulmonary oedema may also occur in patients with incipient cardiac failure, when large doses of contrast medium, particularly of the high osmolar type, are used [2, 29–31]. Pulmonary oedema is reported to be seen in 10–20% of cases of fatal reactions to intravenous infusion of RCM. Subclinical pulmonary oedema without obvious signs or symptoms of respiratory distress is thought to be common after intravascular use of RCM but the true incidence is difficult to establish [29–31]. The direct effects of RCM on pulmonary blood vessels and PVR are discussed below.

Effects of RCM on tension of small pulmonary blood vessels

The pulmonary arteries have comparatively little muscle, and possess low intrinsic tone. The response of pulmonary arteries to vasoactive substances may be different from muscular systemic arteries. Indeed, large conduit muscular pulmonary arteries behave differently from small poorly muscularized peripheral arteries [44–46]. The study of isolated small pulmonary arteries in the myograph allowed the investigation of the direct effects of RCM on these blood vessels independent of systemic influences [46]. The results of this study have shown that the effect of RCM on pulmonary arteries is biphasic, consisting of an initial transient vasorelaxation phase followed by a more sustained phase of vasoconstriction. Ionic RCM were more vasoactive in comparison with non ionic media [46].

It seems that both osmolality and the chemical structure of RCM determine the effect of RCM on vascular tension [46, 50–53]. An osmolality above 600 mosmol kg^{-1} H_2O may pull water out of muscle cells, thereby decreasing size and increasing cell electrolyte concentration, leading to hyperpolarization and vasorelaxation [50]. However, vasodilatation is not entirely dependent on high osmolality since it has been shown that the low osmolar ionic dimer ioxaglate can induce marked vasodilatation of the pulmonary arteries. Furthermore, the vasodilatory effect of ioxaglate is greater than that of iopromide (low osmolar non-ionic monomer) although the osmolality both agents is similar [46]. It has been suggested that ionic contrast media are effective vasodilators in comparison with non ionic agents because they can inhibit the influx of calcium ions into the smooth muscle cells which is responsible for the contraction of these cells more efficiently than non ionic media [45, 46].

The mechanisms responsible for the vasoconstrictor effect of RCM are poorly understood and may be due to a direct effect on vascular smooth muscle, neural reflexes or through the modulation of the release of endogenous vasoactive mediators such as ET, histamine, serotonin or nitric oxide, bradykinin, prostaglandins, antidiuretic hormone (ADH), atrionatriuretic peptide (ANP), angiotensin II and renin [14–16, 19, 26, 44–46]. As yet there is no conclusive evidence to support the involvement of these substances in mediating the effects of RCM on the tension of the pulmonary arteries [45, 46]. However, in the kidney, the endogenous vasoconstrictor ET plays an important role in mediating the increase in renal vascular resistance induced by RCM [26, 47].

Effects of RCM on PVR

The isolated blood perfused rat lung is a useful model to investigate the direct effects of RCM on both the normotensive and hypertensive pulmonary vascular bed, independent of systemic stimuli or changes in the cardiac output [44]. Pulmonary hypertension can be induced by exposing the rat to hypoxia for 3 weeks to 4 weeks [44]. The chronic hypoxic model is comparable with the clinical scenario of chronic pulmonary hypertension in which there are structural changes in the pulmonary arteries. It is more relevant than the widely used experimental model of acute pulmonary hypertension secondary to multiple emboli in which structural changes are absent [34, 36]. In chronic pulmonary hypertension the structural changes include an increase in the thickness of the muscle wall of the small pulmonary blood vessels owing to newly developed muscle cells, which exaggerate the reactivity of the blood vessels to vasoactive stimuli [44]. Patients with pulmonary arterial hypertension are known to be vulnerable to the administration of RCM into the pulmonary circulation [34, 35, 37, 38].

In the isolated blood perfused lung of the normal rat, increasing doses of RCM (iotrolan, iopromide, ioxaglate and diatrizoate) and hypertonic solutions of mannitol caused an overall rise in Ppa reflecting an increase in the PVR. The maximum increase in Ppa was observed with the ionic dimer ioxaglate and the lowest was with the non ionic monomer iopromide [44]. In the isolated lungs from chronically hypoxic rats, where baseline Ppa and resistance is high slow rise in Ppa was observed in response to the tested RCM (ioxaglate, iotrolan and iopromide) [44]. The rise in the Ppa observed with ioxaglate was comparable with that of iotrolan but significantly greater than that with iopromide [44]. The increase in PVR induced by RCM is most likely due to a combination of active vasoconstriction of the pulmonary arteries, pulmonary oedema and possible increase in blood viscosity [44–46]. The latter could be secondary to cellular effects (increased aggregation of red blood cells with non ionic media and rigidity with high osmolar solutions) and the high viscosity of some of the contrast agents [41, 44, 45, 49, 54]. Contrast media may also activate adhesion of leucocytes to the endothelium causing capillary plugging and stasis of red blood cells in the small vessels thereby precipitating an increase in vascular resistance [44].

Pulmonary oedema produced by RCM could also be responsible for the increase in the PVR and rise in Ppa. It is possible that ioxaglate induced the largest increase in PVR of the isolated rat lung preparation because of a combination of vasoconstriction and pulmonary oedema. Experimental studies have shown that ioxaglate can induce vasoconstriction of the small pulmonary arteries and is more cytotoxic to the vascular endothelium in comparison with diatrizoate and non ionic media (Table 1) [46, 55–57]. The extent of ioxaglate induced pulmonary oedema in the rat was four fold higher in comparison with the non ionic monomer iohexol [57]. It is of interest that in the rat nitric oxide [55] and oestrogen [56] offered some protection against ioxaglate induced pulmonary oedema.

Surprisingly, the iso-osmolar contrast agent iotrolan, which has the lowest vasoactivity, induced a significant increase in the PVR of the isolated blood perfused lung of both the normal and chronic hypoxic rat [44]. High viscosity and rheological effects on red blood cells of iotrolan could be responsible for the observed increase in the vascular resistance of the isolated lung preparation, which is perfused with blood (Table 1) [44–46]. The non ionic monomer iopromide had the least effects on PVR of both the normotensive and hypertensive rat lung preparation [44]. This is understandable since iopromide has low vasoactive properties, low viscosity and its effects on the endothelium are minimal to cause pulmonary oedema leading to an increase in the PVR. [46, 57, 58]. Clinical experience has also shown the absence of major haemodynamic effects with the use of low osmolar non ionic monomers in pulmonary angiography even in patients with pulmonary hypertension [59, 60].

In summary, RCM can induce an increase in PVR and rise in Ppa through direct effects on the pulmonary circulation. The least changes are observed with the non ionic monomers. The mechanisms responsible for the rise in Ppa remain poorly defined.

Pulmonary effects of intrabronchial aspiration of water soluble RCM

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Intrabronchial administration of high osmolar water soluble RCM is dangerous and can induce severe bronchial irritation and pulmonary oedema [2, 61, 62]. On the other hand low osmolar RCM are well tolerated by the lungs following aspiration with minimal histological reaction [63, 64]. These agents should be used for upper gastrointestinal tract imaging whenever there is the possibility of aspiration [63, 64]. In addition, the safe use of the non ionic iso-osmolar dimer iotrolan in bronchoscopic bronchography has been described [65–71]. Nausea, vomiting, coughing and headache were the commonly reported side effects of this procedure. Urticaria and fever were rare. Life threatening adverse events or significant changes in the percentage of arterial oxygen saturation were not observed [65–71].

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References

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References

1. Ansell G, Tweedle MC, West CR, Evans P, Couch L. The current status of reactions to intravenous contrast media. Invest Radiol 1980;15:S32–S39.[Medline]
2. Morcos SK. Radiological contrast media. In: Meyler's Side Effects of Drugs (14th edn). Dukes MNG, Aronson JK, editors. Elsevier Science B. V., 2000:1596–630.
3. Littner MR, Rosenfield AT, Ulreich S, Putman CE. Evaluation of bronch-spasm during excretory urography. Radiology 1977;124:17–21.[Abstract]
4. Littner MR, Ulreich S, Putman CE, Rosenfield AT, Meadows G. Bronchospasm during excretory urography: lack of specificity for the methyl glucamine. AJR Am J Roentgenol 1981;137:477–81.[Medline]
5. Dawson P, Pitfield J, Britain J. Contrast media and bronchospasm: a study with iopamidol. Clin Radiol 1983;34:227–30.[Medline]
6. Longstaff AJ, Henson JHL. Bronchospasm following intravenous injection of ionic and non ionic low osmolality contrast media. Clin Radiol 1985;36:651–3.[Medline]
7. Wilson ARM, Davis P. Ventilatory function during urography: a comparison of iopamidol and sodium iosalamate. Clin Radiol 1988;39:490–3.[Medline]
8. Cipolla P, Castano M, Kirchin MA, de Haen C, Tirone P. Effects of iodinated contrast media on pulmonary airway resistance in anesthetized guinea pigs. Acad Radiol 1995;2:306–12.[Medline]
9. Laude EA, Emery CJ, Suvarna SK, Morcos SK. The effect of antihistamine, endothelin antagonist and corticosteroid prophylaxis on contrast media induced bronchospasm. Br J Radiol 1999;72:1058–63.[Abstract/Free Full Text]
10. Lasser EC, Lyon SG, Berry CC. Reports on contrast media reactions: analysis of data from reports to the U.S. Food and Drug Administration. Radiology 1997;203:605–10 (erratum 876).[Abstract]
11. Seyferth W, Zeitler E. Pulmonary angiography: comparison of cough stimulation effects of diatrizoate and ioxaglate. Radiology 1987;164:875–6.[Medline]
12. Greenberger PA, Patterson R. The prevention of immediate generalized reactions to

radiocontrast media in high-risk patients. J Allergy Clin Immunol 1991;87:867–72.[Medline]
13. Laroche D, Aimone-Gastin, Dubois F, et al. Mechanisms of severe immediate reactions to iodinated contrast material. Radiology 1998;209:183–90.[Abstract]
14. Assem ES, Bray K, Dawson P. The release of histamine from human basophils by radiological contrast agents. Br J Radiol 1983;56:647–52.[Abstract]
15. Peachell P, Morcos SK. Effect of radiographic contrast media on histamine release from human mast cells and basophils. Br J Radiol 1998;71:24–30.[Abstract/Free Full Text]
16. Szolar DH, Saeed M, Flueckiger F, et al. Effects of Iopromide on vasoactive peptides and allergy-mediated substances in healthy volunteers. Invest Radiol 1995;30:144–9.[Medline]
17. Szolar DH, Saeed M, Flueckiger F, et al. Response of vasoactive peptides to a non-ionic contrast media in patients undergoing pulmonary angiography. Invest Radiol 1995;30:511–6.[Medline]
18. Lasser EC, Walter A, Reuter SR, Lang I. Histamine release by contrast media. Radiology 1971;100:683–6.[Medline]
19. Ring J, Sovak N. Release of serotonin from human platelets in vitro by radiographic contrast media. Invest Radiol 1981;16:245–8.[Medline]
20. Lasser EC. Adverse reactions to intravascular administration of contrast media. Allergy 1981;36:369–73.[Medline]
21. Lasser EC, Berry CC, Lee B, Talner LB, Lewis C, Santani LC, et al. Pretreatment with corticosteroids to alleviate reactions to intravenous contrast material. N Engl J Med 1987;317:845–9.[Abstract]
22. Lasser EC. Pretreatment with corticosteroids to prevent reactions to IV contrast material: overview and implications. AJR Am J Roentgenol 1988;150:257–9.[Medline]
23. Lasser EC, Berry CC, Mishkin MM, Williamson B, Zheutlin N, Silverman JM. Pretreatment with corticosteroids to prevent adverse reactions to nonionic contrast media. AJR Am J Roentgenol 1994;162:523–6.[Abstract]
24. Dawson P, Sidhu PS. Is there a role for corticosteroid prophylaxis in patients at increased risk of adverse reactions to intravascular contrast agents? Clin Radiol 1993;48:225–6. [Medline]
25. Wolf GL, Mishkin MM, Roux SG, Halpern EF, Gottlieb J, Zimmerman J, et al. Comparison of the rates of adverse drug reactions. Ionic agents, ionic agents combined with steroids and non-ionic agents. Invest Radiol 1991;26:404–10.[Medline]
26. Oldroyd SD, Morcos SK. Endothelin: what does the radiologist need to know? Br J Radiol 2000;73:1246–51.[Abstract/Free Full Text]
27. Laude EA, Emery CJ, Morcos SK. Ventilatory effects of radiographic contrast media. Br J Radiol 1998;71:1143–8.[Abstract/Free Full Text]
28. Morettin LB, Zhan X, Sant Ambrogio FB, Sant Ambrogio G. Cardiorespiratory responses elicited by right atrial injections of iodinated contrast media. Invest Radiol 1994;29:201–9. [Medline]
29. Hauggaard A. Non-cardiogenic pulmonary oedema after intravenous administration of non ionic contrast media. Acta Radiol 1996;37:823–5.[Medline]
30. Paul RE, George G. Fatal non-cardiogenic pulmonary oedema after intravenous non-ionic radiographic contrast. Lancet 2002;359:1037–8.[CrossRef][Medline]
31. Frisinger G, Schaffer J, Criley M, Gartner R, Ross J. Haemodynamic consequences of the injection of radiopaque material. Circulation 1965;31:730–40.
32. Mills SR, Jackson BF, Older RA, Heaston DK, Moore AV. The incidence, aetiologies and avoidance of complications of pulmonary angiography in a large series. Radiology 1980;136:295–9.[Abstract]
33. Peck WW, Slutsky RA, Hackney DB, et al. Effects of contrast media on pulmonary hemodynamics: comparison of ionic and non-ionic agents. Radiology 1983;149:371–4. [Abstract]
34. Schrader R, Hellige G, Kaltenbach M, Kober G. The haemodynamic side-effects of ionic and non-ionic contrast media in the presence of pulmonary hypertension: experimental and

clinical investigation. Eur Heart J 1987;8:1322–31.[Abstract]
35. Nicod P, Peterson K, Levine M, Dittrich H, Buchbinder M, Chappuis L, et al. Pulmonary angiography in severe chronic pulmonary hypertension. Ann Intern Med 1987;107:565–8. [Medline]
36. Rees CR, Palmaz JC, Garcia O, Alvarado R, Siegle RL. The hemodynamic effects of the administration of ionic and non ionic contrast material into the pulmonary arteries of a canine Model of acute pulmonary hypertension. Invest Radiol 1988;23:184–9.[Medline]
37. Tajima H, Kumazaki T, Tajima N, Murakami R, Gemma K. Effect of iohexol on pulmonary arterial pressure at pulmonary angiography in patients with pulmonary hypertension. Radiat Med 1994;12:197–9.[Medline]
38. Pitton MB, Duber C, Mayer E, Thelen M. Hemodynamic effects of nonionic contrast bolus injection and oxygen inhalation during pulmonary angiography in patients with chronic major vessel thromboembolic pulmonary hypertension. Circulation 1996;94:2485–91. [Abstract/Free Full Text]
39. Almen T, Aspelin P, Nilsson P. Aortic and pulmonary arterial pressure after injection of contrast media into the right atrium of the rabbit. Acta Radiol 1980;(Suppl. 362): 37–41.
40. Sunnegardh O, Heitala SO, Wierell S, et al. Systemic, pulmonary and renal haemodynamic effects of intravenously infused lopental, A comparison in the pig of a new low osmolar non-ionic medium with saline and iohexol. Acta Radiol 1990;31:395–9.[Medline]
41. Sorenson L, Sunnegardh O, Svanegard J, Lundquist S, Heitala SO. Systemic and pulmonary haemodynamic effects of intravenous infusion of non-ionic isoosmolar dimeric contrast media. An investigation in the pig of two ratio 6 contrast media. Acta Radiol 1994;35:383–90.[Medline]
42. Kuhtz-Buschbeck JP, Ehrhardt K, Kohnlein S, Radtke W, Heintzen P. Gadopentetate dimeglumine and iodinated contrast media. Haemodynamic side effects after bolus injection in pigs. Invest Radiol 1997;32:111–9.[CrossRef][Medline]
43. Schrader R, Wolpers HG, Korb H, Hoeft A, Klepzig H, Kober G, et al. Central venous injection of large amounts of contrast media—advantages of low osmolar contrast medium in experimentally induced pulmonary hypertension. Z Kardiol 1984;73:434–41.[Medline]
44. Emery CJ, Fang L, Laude EA, Morcos SK. Effects of radiographic contrast media on pulmonary vascular resistance of normoxic and chronically hypoxic pulmonary hypertensive rats. Br J Radiol 2001;74:1109–17.[Abstract/Free Full Text]
45. Morcos SK, Dawson P, Pearson JD, et al. The haemodynamic effects of iodinated water soluble radiographic contrast media: a review. Eur J Radiol 1998;29:31–46.[CrossRef] [Medline]
46. Wang YX, Emery CJ, Laude E, Morcos SK. Effects of radiocontrast media on the tension of isolated small pulmonary arteries. Br J Radiol 1997;70:1229–38.[Abstract/Free Full Text]
47. Morcos SK. Contrast media-induced nephrotoxicity-questions and answers. Br J Radiol 1998;71:357–65.[Abstract/Free Full Text]
48. Dawson P, Harrison MJ, Weisblatt E. Effects of contrast media on red cell filterability and morphology. Br J Radiol 1983;56:707–10.[Abstract]
49. Liss P, Nygren A, Olsson U, Ulfendahl HR, Erikson U. Effects of contrast media and mannitol on renal medullary blood flow and red cell aggregation in the rat kidney. Kidney Int 1996;49:1268–75.[Medline]
50. Gomi N. Vasoconstriction by angiographic contrast media in isolated canine arteries. Br J Radiol 1992;65:961–7.[Abstract]
51. Karstoft J, Baath L, Jansen I, et al. Vasoconstriction of isolated arteries induced by angiographic contrast media. A comparison of ionic and non-ionic contrast media iso-osmolar with plasma. Acta Radiol 1995;36:312–6.[Medline]
52. Haylor JL, El Sayed AA, El Nahas AM, Morcos SK. The effect of sodium iothalamate on the vascular resistance of isolated perfused rat kidney. Br J Radiol 1991;64:50–4.[Abstra
53. Pugh ND, Hutcheson IR, Edwards DH, et al. Angiographic contrast media relax isolated rabbit aorta through an endothelium-independent mechanism that may not depend on th

presence of the iodine atom. Br J Radiol 1995;68:23–6.[Abstract]
54. Spitzer S, Munster W, Sternitzky R, Bach R, Jung F. Influence of iodixanol-270 and iopentol-150 on the microcirculationin man: influence of viscosity on capillary perfusion. Clin Hemorheol 1999;20:49–55.
55. Sendo T, Kataoka Y, Takeda Y, Furuta W, Oishi R. Nitric oxide protects against contrast media-induced pulmonary vascular permeability in rats. Invest Radiol 2000;35:427–8.
56. Tominaga K, Kataoka Y, Sendo T, Furuta W, Niizeki M, Oishi AR. Contrast media-induced pulmonary vascular hyperpermeability is aggravated in a rat climacterium model. Invest Radiol 2001;36:131–5.[CrossRef][Medline]
57. Zhang H, Holt CM, Malik N, Shepherd L, Morcos SK. Effects of radiographic contrast media on proliferation and apoptosis of human vascular endothelial cells. Br J Radiol 2000;73:1034–41.[Abstract/Free Full Text]
58. Thomsen HS, Morcos SK. Radiographic contrast media. BJU International 2000;86 Suppl. 1:1–10.
59. Zuckerman DA, Sterling KM, Oser RF. Safety of pulmonary angiography in the 1990s. J Vasc Interv Radiol 1996;7:199–205.[Abstract]
60. Nilsson T, Carlsson A, Mare K. Pulmonary angiography: a safe procedure with modern contrast media and technique. Eur Radiol 1998;8:86–9.[CrossRef][Medline]
61. Trulzsch DV, Penmetsa A, Karim A, Evans DA. Gastrografin-induced aspiration pneumonia: a lethal complication of computed tomography. South Med J 1992;85:1255–6. [Medline]
62. Donnely LF, Frush DP, Frush KS. Aspirated contrast material contributing to respiratory arrest in a pediatric trauma patient. AJR Am J Roentgenol 1998;171:471–3.[Medline]
63. Ginai AZ, Ten Kate FJW, Ten Berg RGM, Hoornstra K. Experimental evaluation of various available contrast agents for use in the upper gastrointestinal tract in case of suspected leakage. Effects on lungs. Br J Radiol 1984;57:895–901.[Abstract]
64. Ginai GAZ, Bubberman A, Zondervan PE, et al. The histological response of the lungs of rats to potentially suitable water soluble bronchographic contrast agents iotrolan (a non-ionic dimer) and iopamidol (a non-ionic monomer). Br J Radiol 1993;66:773–7.[Abstract]
65. Morcos SK, Baudouin SV, Anderson PB, Beedie R, Bury RW. Iotrolan in selective bronchography via the fibreoptic bronchoscope. Br J Radiol 1989;62:383–5.[Medline]
66. Morcos SK, Anderson PB, Ward P, Weber S, Wenzel-Hora BI. The efficacy of bronchography via the flexible bronchoscope using a water soluble non-ionic dimer (iotrolan) in diagnosing airways diseases. J Bronchol 1996;3:106–11.
67. Morcos SK, Anderson PB, Baudouin SV, et al. Suitability of and tolerance to iotrolan300 in bronchography via the fibreoptic bronchoscope. Thorax 1990;45:628–9.[Abstract]
68. Riebel T, Wartner R. Use of non-ionic contrast media for tracheobronchography in neonates and young infants. Eur J Radiol 1990;11:120–4.[Medline]
69. Morcos SK, Anderson PB, Kennedy A. Bronchography with iotrolan300 via the flexible bronchoscope in the evaluation of focal lung opacity. J Bronchol 1994;1:112–5.
70. Morcos SK, Anderson PB. Airways and lung: bronchography through the fiberoptic bronchoscope. Radiology 1996;200:612–4.[Medline]
71. Rogers TK, Kennedy A, Anderson P, Morcos SK. Role of bronchography with iotrolan in the diagnosis of obliterative bronchiolitis after heart-lung transplantation. J Bronchol 1998;5:53–6.

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United States Patent *6,875,887*
Lorenzini , et al. **April 5, 2005**

Process for the preparation of iopamidol

Abstract

A compound and salts and enantiomers thereof, having the formula: ##STR1## wherein R7 is an acyl group.

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References Cited [Referenced By]

U.S. Patent Documents

4001323	Jan., 1977	Felder et al.	
4352788	Oct., 1982	Felder et al.	
4396598	Aug., 1983	Lin.	
4997983	Mar., 1991	McCarthy	564/153.
5177261	Jan., 1993	McCarthy et al.	
5204005	Apr., 1993	Doran, III et al.	
5256393	Oct., 1993	McCarthy et al.	
5362905	Nov., 1994	Villa et al.	
5371278	Dec., 1994	McCarthy et al.	
5396003	Mar., 1995	McCarthy	570/262.
5527926	Jun., 1996	Ranganathan et al.	
5550287	Aug., 1996	Cannata et al.	

Foreign Patent Documents

0118347	Sep., 1984	EP.
0516050	Dec., 1992	EP.
2272218	May., 1994	GB.
WO 92/14539	Sep., 1992	WO.
WO 92/18464	Oct., 1992	WO.
WO 95/04031	Feb., 1995	WO.

Other References

Grainger & Dawson, "Low Osmolar Contrast Medis: An Appraisal", Clinical Radiology, 42:1-5 (1990).
Pillai, et al., "Heterocyclic Nonionic X-ray Contrast Agents. 3. The Synthesis of 5-[4 (Hydroxymethyl)-2-oxo-3-oxazolidinyl]-2,4,6-triiodo-1,3-benzene-dicarb oxamide Derivatives" J. Org. Chem, 59:1344-1350 (1994).
"Iopamidol", The Merck Index, 11th Edition, 4943-4944 (1989).
Clerici et al., "Mass Spectral Characterization of Iopamidol", Biomedical Mass Spectrometry, vol. 9, No. 6 pp 257-265 (1982).
Anelli et al., Tetrahedron, vol. 53, No. 34, pp 11919-11928.

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Parent Case Text

This application is a divisional of U.S. application Ser. No. 09/257,690, filed Feb. 26, 1999, now U.S. Pat. No. 6,803,485, the entire contents of which are hereby incorporated by reference.

Claims

What is claimed is:

1. A compound and salts and enantiomers thereof, having the formula: ##STR6##

wherein R.sup.7 is an acyl group.

2. The compound according to claim 1, wherein each R.sup.7 is independently selected from the group consisting of formyl, acetyl, propionyl, butanoyl, pivaloyl, pentanoyl, trifluoroacetyl, trichloroacetyl and benzoyl.

3. The compound according to claim 2, wherein each R.sup.7 is independently selected from the group consisting of formyl, acetyl, propionyl and butanoyl.

4. The compound according to claim 3, wherein each R.sup.7 is acetyl.

Description

TECHNICAL FIELD

The present invention relates to a process for the preparation of nonionic, water soluble compounds that are useful as contrast agents.

BACKGROUND OF THE INVENTION

The introduction in X-ray diagnosis of contrast media containing non-ionic iodinated compounds as opacifying agents represented a remarkable progress in the state of the technique, so far that, these media will eventually substitute the traditional iodinated ionic products (see Grainger and Dawson, Clinical Radiology, 1990, 42, 1-5). These nonionic compounds, such as, (S)-N,N'-bis[2-hydroxy-1-(hydroxy(methyl)ethyl-5-[(2-hydroxy-1-oxypropylam ino]-2,4,6,-triiodo-1,3-benzenedicarboxamide (iopamidol) and 5-[acetyl(2,3-dihydroxypropyl)amino]-N,N'-bis[2,3-dihydroxypropyl]-2,4,6,-triiodo-1,3-benzenedicarboxamide (iohexol), are useful as contrast enhancing agents for X-ray, magnetic resonance imaging (MRI) and angiography. These compounds have a lower frequency of adverse reactions in patients, during intravenous injection, than many ionic contrast agents.

However, the synthetic processes and, particularly, the final purification of these products are complex and expensive. Neutral iodinated opacifying agents differ from ionic ones because they cannot be isolated and purified by precipitation from water due to their high solubility. Thus the following problems must be solved: the removal of ionic species, usually inorganic salts, from the final reaction mixture, the recovery of valuable reagents in excess and of water-soluble reaction media. A preferred technique to be performed (see for example, U.S. Pat. Nos. 4,352,788 and 4,001,323) is the one based on the submission of operations such as:

preliminary removal of the solvent,

extraction of the residual reaction medium, preferably with a chlorinated solvent,

elution of the aqueous phase on a system of columns of cationic and anionic ion-exchange resins,

concentration of the elute by evaporation,

crystallization of the crude residue.

The drawbacks related to this type of process include: a) a requirement for large complex and expensive purification plants for ion-exchange resins; b) a large quantity of thermal energy is required for the concentration of the water employed; c) the concentration of extremely diluted solutions causes the corresponding concentration of trace impurities; and d) the final product is exposed to a long-lasting thermal treatment.

U.S. Pat. No. 4,001,323 (the '323 patent) describes a process for preparing iopamidol which involves a) reacting 5-amino-2,4,6-triiodoisophthalyl dichloride (ATIPA-CI) with 2(S)-acetoxypropionyl chloride to form an acetyl-amide intermediate; b) reacting the acetyl amide intermediate with serinol to provide acetyliopamidol; c) reacting the acetyliopamidol with an aqueous base, such as, sodium hydroxide to hydrolyze the ester and provide iopamidol. The product is then purified by ion exchange treatment, followed by recrystallization from ethanol.

U.S. Pat. No. 4,352,788 (the '788 patent) describes a process for preparing compounds similar to the compounds of the '323 patent. The principle difference is the compounds of the '788 patent are alkylated at the aromatic nitrogen atom. The products are isolated by counter-current extraction or by using exchange resins.

However, problems that exist with the process disclosed in the '323 and the '788 patents include a)

the use of a hazardous solvent; b) the basic hydrolysis can induce racemization of the optically active compound and may produce material which does not meet the U.S.P. optical rotation specification for iopamidol.

U.S. Pat. No. 4,396,598 (the '598 patent) discloses a method for preparing N,N'-bis(2,3-dihydroxypropyl)-5-N-(2-hydroxyethyl)glycolamido-2,4,6-triiod oisophthalimide. This patent also discloses the preparation starting with ATIPA-CI. However in the '598 patent, the polyhydroxy product is purified via preparative liquid chromatography.

U.S. Pat. No. 5,550,287 discloses a method for purification of the contrast agents again using a column with a strong anionic resin followed by a column with a weak anionic resin.

U.S. Pat. No. 5,204,005 discloses the use of a reverse phase chromatographic process for purification of water soluble, non-ionic contrast medium compounds.

An object of the present invention is to provide and process to prepare contrast agents which do not racemize the product.

An object of the present invention is to provide and process which furnishes the product contrast agent having a specific rotation that meets the requirements of the U.S.P. specification.

An object of the present invention is to provide an efficient method for the purification of non-ionic water soluble contrast agents.

SUMMARY OF THE INVENTION

The present invention relates to an improved process for the manufacture and purification of contrast enhancing agents, such as, iopamidol and iohexol. The process converts 5-amino-2,4,6-triiodoisophthalyl dichloride (ATIPA-CI) to an isophthaiyl-diamide, such as, for example, 5-amino-N,N'-bis(1,3-diacetoxy-2-propyl)-2,4,6-triiodoisophthalamide(tetra acetyl-diamide) in a single reaction vessel by first reacting the ATIPA-CI with 2 equivalents of a dihydroxy-amine such as, for example, serinol, (2-amino-1,3-dihydroxypropane) or another suitable dihydroxyamino compound, in the presence of triethylamine, followed by treatment with an acid anhydride in the presence of a catalytic amount of dimethylaminopyridine (DMAP), to form the tetraester-diamide. The tetraester-amide product is then treated with an 2(S)-alkanoyloxylated propionyl chloride to produce the pentaester of iopamidol. The pentaester is treated with a catalytic amount of hydrochloric acid in methanol to deacylate the ester and provide iopamidol. The crude product is treated with an acid scavenging resin to remove the acid and purified by passing through a bed of nonionic polymeric adsorbent resin to remove other impurities from the reaction. The final purification is performed by recrystallization from ethanol or a mixture of acetonitrile in ethanol to provide pure iopamidol.

DETAILED DESCRIPTION OF THE INVENTION

All patents, patent applications, and literature references cited in the specification are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present disclosure, including definitions, will prevail.

The present invention relates to a process for the preparation of a polyhydroxy compound and salts and enantiomers thereof having formula I. ##STR2##

wherein R.sup.1 and R.sup.2 are dihydroxyalkyl groups, and R.sup.3 is hydrogen, alkyl, or hydroxy. The process comprising the step of deacylating an acylated compound having the formula: ##STR3##

in an acidic medium, to provide the free polyhydroxy compound. R.sup.4 and R.sup.5 are optionally

acylated dihydroxyalkyl groups and R.sup.6 is lower alkyl. The polyhydroxy compound can be purified by treatment with an acid scavenging resin.

The invention also contemplates compounds having the formula ##STR4##

wherein each R.sup.7 is an acyl group, and salts and enantiomers thereof.

Examples of acyl groups include groups such as, for example, formyl, acetyl, propionyl, butanoyl, pivaloyl, pentanoyl, trifluoroacetyl, trichloroacetyl, benzoyl, and the like. The preferred acyl groups are formyl, acetyl, propionyl, and butanoyl. The most preferred acyl group is acetyl.

The dihydroxyalkyl groups are straight or branched chain alkyl radicals containing from 2 to 6 carbon atoms and having two hydroxy groups. Most preferred dihydroxyalkyl groups are 1,3-dihydroxypropyl, 1,2-dihydroxypropyl.

The lower alkyl groups include straight or branched chain alkyl groups having from 1 to about 6 carbon atoms. Examples of lower alkyl groups include groups such as, for example, methyl, ethyl, n-propyl, iso-propyl, 2-methylpropyl n-butyl, 2-butyl, t-butyl n-pentyl, 1-methylbutyl, 2,2-dimethylbutyl, 2-methylpentyl, 2,2-dimethylpropyl, and n-hexyl. The preferred lower alkyl groups are methyl, ethyl, n-propyl, iso-propyl, n-butyl, and t-butyl. More preferred are methyl and ethyl. Most preferred is methyl.

The advantages of the present invention include reduction of racemization of the product and an improved method for isolation of the product. This provides a product with a higher enantiomeric excess (ee) than the methods disclosed in the documents discussed above. The process of the invention involves the deacylation of an ester of iopamidol using a catalytic amount of acid. The acid is removed by batch treatment with a small amount of an acid scavenging resin. Final purification involves passing an aqueous solution of the product through a column of non-ionic polymeric adsorbent resin, followed by concentration to an oil and recrystallization from acetonitrile/ethanol or ethanol alone. This process consistently produces material which meets all U.S.P. specifications including the optical rotation specification.

Typical acid scavenging resins include weak basic resins such as, for example, IRA-68, IRA-67, Dowex.RTM. WGR-2, and the like. These resins remove any acid present.

Typical nonionic polymeric adsorption resins include polyaromatic resins, such as, for example, Amberlite XAD-16, XAD-4, and the like. These resins function to remove impurities formed during the reaction process.

A preferred embodiment is illustrated in Scheme 1 below. The process converts 5-amino-2,4,6-triiodoisophthalyl dichloride (ATIPA-CI) to 5-amino-N,N'-bis(1,3-diacetoxy-2-propyl)-2,4,6-triiodoisophthalamide(tetra acetyl-diamide) in a single reaction vessel by first reacting ATIPA-CI with 2 equivalents of serinol in the presence of triethylamine followed by treatment with acetic anhydride in the presence of a catalytic amount of dimethylaminopyridine (DMAP). The tetraacetyl-diamide product is readily isolated by precipitation from water and further purification is generally not required. The tetraacetyl compound is treated with 2(S)-acetoxypropionyl chloride to provide a pentaacetyl-triamide. The acetate groups are removed by a transesterification reaction with hydrochloric acid in methanol to provide iopamidol. The acid is removed with an acid scavenging resin. Other impurities are removed using a polymer absorption resin. The product can be crystallized from ethanol or, optionally, if it contains excessive impurities, an acetonitrile/ethanol mixture. ##STR5##

Preferred compounds of the invention include the compounds:

(S)--N,N'-bis[2-hydroxy-1-(hydroxy(methyl)ethyl]-5-[(2-hydroxy-1-oxypropyl- amino]-2,4,6,-

triiodo-1,3-benzenedicarboxamide,

(S)--N,N'-bis[2,3-dihydroxypropyl]-5-[(2-hydroxy-1-oxypropylamino]-2,4,6,-t riiodo-1,3-benzenedicarboxamide, and

5-[acetyl(2,3-dihydroxypropyl)amino]-N,N'-bis[2,3-dihydroxypropyl]-2,4,6,-t riiodo-1,3-benzenedicarboxamide, (iohexol).

The process of the invention includes a method for deacylating a compound wherein all of the hydroxy groups have been acylated and a method for deacylating monoacylated compounds such as, for example, acetyliopamidol. Examples of the alkanoyloxy group include acetyloxy, propionyloxy, butanoyloxy and the like. A preferred alkanoyloxy group is acetyloxy. The acyl groups include groups such as, for example, acetyl, propionyl, butanoyl and the like. A preferred acyl group is acetyl.

The invention also contemplates a method for the purification of water soluble nonionic contrast agents.

As used herein, the term "acyl" refers to groups having the formula --C(.dbd.O)--R.sup.95 wherein R.sup.95 is hydrogen or a lower alkyl or aryl group. Representative examples of acyl groups include groups such as, for example, formyl, acetyl, propionyl, and the like.

As used herein, the term "alkyl" refers to straight or branched chain alkyl radicals containing from 1 to 12 carbon atoms. The term "lower alkyl" refers to straight or branched chain alkyl radicals containing from 1 to 6 carbon atoms including, but not limited to, methyl, ethyl, n-propyl, iso-propyl, 2-methylpropyl n-butyl, 2-butyl, t-butyl n-pentyl, 1-methylbutyl, 2,2-dimethylbutyl, 2-methylpentyl, 2,2-dimethylpropyl, n-hexyl, and the like.

As used herein, the term "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. Aryl groups can be unsubstituted or substituted with one, two or three substituents.

As used herein, the term "dihydroxyalkyl" refers to straight or branched chain alkyl radicals containing from 2 to 6 carbon atoms and having two hydroxy groups. Representative examples of dihydroxyalkyl groups include groups such as, for example, 1,3-dihydroxypropyl, 1,2-dihydroxypropyl, and the like.

The term "halo" as used herein refers to F, Cl, Br or I.

The term "haloalkyl" as used herein refers to a lower alkyl group in which one or more hydrogen atoms has been replaced with a halogen including, but not limited to, trifluoromethyl, trichloromethyl, difuoromethyl, dichloromethyl, fluoromethyl, chloromethyl, chloroethyl, 2,2-dichloroethyl and the like.

As used herein, the terms "S" and "R" configuration are as defined by the IUPAC 1974 Recommendations for Section E, Fundamental Stereochemistry, Pure Appl. Chem. (1976) 45, 13-30.

The reagents required for the synthesis of the compounds of the invention are readily available from a number of commercial sources such as Aldrich Chemical Co. (Milwaukee, Wis., USA); Sigma Chemical Co. (St. Louis, Mo., USA); and Fluka Chemical Corp. (Ronkonkoma, N.Y., USA); Alfa Aesar (Ward Hill, Mass. 01835-9953); Eastman Chemical Company (Rochester, N.Y. 14652-3512); Lancaster Synthesis Inc. (Windham, N.H. 03087-9977); Spectrum Chemical Manufacturing Corp. (Janssen Chemical) (New Brunswick, N.J. 08901); Pfaltz and Bauer (Waterbury, Conn. 06708).

Compounds which are not commercially available can be prepared by employing known methods from the chemical literature.

The polymeric resins, e.g., IR-68 and Ambelite XAD-16 are available from suppliers such as Rohm and Haas Company (Philadelphia, Pa. 19106)

The following examples illustrate the process of the invention, without limitation.

EXAMPLE 1

N,N'-bis[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]-5-amino-2,4,6-triiodo-1, 3-benzenedicarboxamide

A suitable reaction vessel was charged with 50 kg of 5-amino-2,4,6-triiodoisophthalyl dichloride (ATIPA-CI) and 75 kg dimethylacetamide (DMA) and mixed. A solution of 18.5 kg of 2-amino-1,3-propanediol (serinol) and 30 kg of triethylamine in 45 kg of DMA was added to the above vessel. The reaction was mixed while gradually elevating the temperature to about 30.degree. C. This temperature was maintained for about 1.5 hours. The reaction was cooled and 0.5 kg of 4-dimethylaminopyridine was added to the vessel followed by the slow addition of 52 kg of acetic anhydride. The reaction was stirred for about 2 hours and quenched by slow addition to water. The solid was isolated by filtration, washed with water and dried (yield: 66 kg; 90%). .sup.1 H NMR (300 MHz, DMSO-d.sub.6) .delta. 2.0 (S, 12H), 4.1(m, 8H), 4.3(m, 2H), 5.5 (S, 2H), 8.4, 8.7 (2d, 2H). .sup.13 C NMR (75 MHz, DMSO-d.sub.6) .delta. 20.8, 46.9, 62.1, 73.5 79.7, 147.6, 148.5, 169.5, 170.2.

EXAMPLE 2

Preparation of Pentaacetyliopamidol

The product, 55 kg, prepared in Example 1, was dissolved in 60 kg of DMA. 2(S)-Acetoxypropionyl chloride, 20 kg, was added slowly. The reaction was stirred at room temperature for about 2 hours and quenched by the slow addition of isopropanol. The mixture was neutralized with tributylamine. The pentaacetyliopamidol is collected by filtration, washed with isopropanol and dried (yield: 56 kg, 90%). .sup.1 H NMR (300 Mz, DMSO-d.sub.6) .delta. 1.5 (d, 3H), 2.0 (S, 12H), 2.1(2S, 3H0, 4.1(m, 8H), 4.3(m, 2H), 5.2(q, 1H), 8.8 (d, 1H0, 8.9(t, 1H), 10.1(S, 1H). .sup.13 C NMR (75 MHz, DMSO-d.sub.6) 17.6, 20.8, 47.0, 62.1, 69.4, 90.1, 99.0, 142.4, 149.6, 168.0, 169.1, 169.5, 170.3.

EXAMPLE 3

Preparation of Iopamidol

A solution of 58 kg of pentaacetyliopamidol in 400 L of methanol containing a catalytic amount, 400 g, of aqueous hydrochloric acid was heated at reflux for about 30 hours. The methanol was removed by distillation and the residue dissolved in water. The acid was neutralized by stirring the solution with an acid-scavenging resin (IRA-68). The resin was removed by filtration and the resulting aqueous solution was passed through a 50 kg column of amberlite XAD-16 resin. The eluant was concentrated to provide an oil and the residue crystallized by heating the oil in a mixture comprising 40 kg of acetonitrile and 150 L of ethanol, followed by cooling. The iopamidol was collected by filtration, washed with ethanol and dried (yield: 34 kg, 74%).

Specific Rotation [.alpha.].sub.D.sup.20 =-5.0 in methanol. .sup.1 H NMR (300 MHz, D.sub.2 O) .delta. 1.6(d, 3H), 3.8 (d, 8H), 4.2(m, 2H), 4.5(q, 1H). 13C NMR (75 MHz, D.sub.2 O) .delta. 21.5, 55.1, 61.8, 70.2, 91.0, 99.8, 144.2, 151.2, 173.8, 178.6.

Comparative Example 1

L-5-(.alpha.-Acetoxypropionylamino)2,4,6-triiodo-isophthalyl chloride

A solution of 100 g (168 mmole) 5-amino-2,4,6-triiodo-isophthalyl chloride, in 100 ml of dimethylacetamide was prepared. L-2-Acetoxypropionyl chloride was added dropwise to the solution at room temperature. The mixture was stirred for 16 hours, at ambient temperature. The reaction mixture was diluted with 200 mL of acetone and added dropwise to 500 mL of cold water. The solid product was collected, washed with water and dried under vacuum at 65.degree. C. (Yield: 110 g, 93

Comparative Example 2

L-5-.alpha.-Acetoxypropionylamino-2,4,6-triiodo-isophthalic acid di-(1,3-dihydroxyisopropylamide) (acetyliopamidol)

The intermediate prepared in Example 1, (27.0 g 38.0 mmole), was dissolved in 140 ml dimethylacetamide. Tributylamine, (14.2 g, 76.6 mmole) was added followed by a solution of 1,3-dihydroxy (8.6 g, 94.4 mmole), in 80 mL of dimethylacetamide. The mixture was stirred and heated at 50 C. for 22 hours. The reaction mixture was added dropwise to 1.0 L of methylene chloride with vigorous agitation, and the resulting precipitate was filtered off and washed to provide 25.8 g of the title compound.

Comparative Example 3

L-5-.alpha.-Hydroxypropionylamino-2,4,6-triiodo-isophthalic acid di-(1,3-dihydroxyisopropylamide) (iopamidol).

The L-5-.alpha.-acetoxypropionylamino-2,4,6,-triiodoisophthalic acid di-(1,3-di-hydroxyisopropylamide) (20 g, 24.4 mmole) was dissolved in water. The pH was adjusted to 11 with concentrated sodium hydroxide solution and heated to 40 C. Additional NaOH solution was added until the pH stabilized, indicating the complete saponification of the acetoxy groups. The reaction mixture was acidified to pH 7 with 3N hydrochloric acid. The resultant solution passed over a column of IR 120 resin (25 g) and followed by passing over a column of A-21 (35 g) resin to desalt the solution. (Resins available from the Rohm & Haas Co.) The product was purified by passing over a XAD-16 column. The title compound was obtained by removal of the solvent in vacuo followed by crystallization from acetonitrile.backslash.water (1:3) (yield: 9.2 g; 48%).

Elemental analysis, calculated for C.sub.17 H.sub.22 I.sub.3 N.sub.3 O.sub.11 : C, 26.27%. and I, 47.79%. Found: C, 26.27% and I, 48.79%. Specific Rotation [.alpha.].sub.D.sup.20 =-4.5 in methanol.

The foregoing is merely illustrative of the invention and is not intended to limit the invention to the disclosed processes and reaction conditions. Variations which are obvious to one of ordinary skill in the art are intended to be included within the scope and nature of the invention which are defined in the appended claims.

* * * * *

REF 16

United States Patent *5,609,851*
Bennani , et al. **March 11, 1997**

Compounds which can be used in contrast products for radiography

Abstract

Polyiodinated nonionic compound that have the formula: ##STR1##

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PCT PUB. Date: **September 2, 1993**

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Intern'l Class: A61K 049/04; C07C 233/05
Field of Search: 424/9.454 564/153

References Cited [Referenced By]

U.S. Patent Documents

4547357	Oct., 1985	Pfeiffer et al.	424/5.
5043152	Aug., 1991	Schaefer et al.	424/5.

Foreign Patent Documents

0049745	Apr., 1982	EP.
0082803	Dec., 1982	EP.

0185130	Mar., 1985	EP.
3429949	Feb., 1986	DE.

Other References

T. Nakanishi, "Hydrophilic Elastomers", Chemical Abstracts, vol. 88, No. 4, Jan. 23, 1978, Abstract No. 24102c.

Primary Examiner: Kumar; Shailendra
Attorney, Agent or Firm: Jacobson, Price, Holman & Stern, PLLC

Claims

We claim:

1. Polyiodinated nonionic compound of formula: ##STR90##

2. Contrast media comprising the compound according to claim 1.

3. Contrast media according to claim 2, comprising an aqueous solution of the compound.

Description

This application is a 371 of PCT/FR93/00175, filed Feb. 22, 1993.

The present invention relates to compounds which can be used in contrast media for radiography.

BACKGROUND

Iodobenzene compounds having on the benzene ring several iodine atoms, in general 3 iodine atoms per benzene ring and various other substituents, have been used for a long time as contrast agents. These other substituents are pharmacologically acceptable groups which allow administration of the compounds to man and to animals. These substituents are generally chosen, on the one hand, to confer on the compounds a sufficient water-solubility for administering these compounds in aqueous solution and, on the other hand, to confer on these compounds sufficient tolerance by the human body.

To this effect, nonionic compounds, that is to say substituted iodobenzenes possessing nonionic substituents, have been proposed.

SUMMARY OF THE INVENTION

The object of the present invention is to provide new nonionic compounds which are well tolerated by the human body, which are stable in aqueous solution, which possess good water-solubility and which, in aqueous solution, possess low viscosity.

To this effect, the present invention provides compounds having formula I: ##STR2## wherein: R.sub.1 represents a group selected from ##STR3## R.sub.4 representing --CH.sub.3, --CH.sub.2 --CH.sub.2 OH, --CH.sub.2 --CHOH--CH.sub.2 OH or --CH--(CH.sub.2 OH).sub.2, ##STR4##

R.sub.4 being as defined above, ##STR5## R.sub.5 representing --H or R.sub.4, ##STR6## R.sub.16 representing R.sub.4 or --CH.sub.2 (CHOH).sub.2 CH.sub.2 OH ##STR7## R.sub.4 being as defined above, ##STR8## representing a linear or branched (C.sub.4 -C.sub.8)alkylene group, a linear or branched hydroxy- or polyhydroxy (C.sub.1 --C.sub.4)alkylene group, a linear or branched (C.sub.1 -C.sub.4)alkoxy(C.sub.4 -C.sub.8)alkylene group, a linear or branched hydroxy- or polyhydroxdroxy(C.sub.1 -C.sub.4)alkoxy(C.sub.4 -C.sub.8)alkylene group,

R.sub.2 represents a group selected from --COR.sub.1 and ##STR9## R.sub.1 being as defined above and R.sub.7 and R.sub.8, which are identical or different, representing a group selected from --H, a linear or branched (C.sub.1 -C.sub.6)alkyl, a linear or branched hydroxy- or polyhydroxy (C.sub.1 -C.sub.6)alkyl group, a linear or branched (C.sub.1 -C.sub.4)alkoxy(C.sub.1 -C.sub.6)alkyl group and a linear or branched hydroxy- or polyhydroxy(C.sub.1 -C.sub.4)alkoxy(C.sub.1 -C.sub.6) alkyl group,

R.sub.3 represents

the group ##STR10## R.sub.9 and R.sub.10 representing R.sub.7 and R.sub.8, a group of formula ##STR11## a group of formula ##STR12## R.sub.10 being as defined above and R.sub.11 having the seine meanings as R.sub.10 except --H.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Polyhydroxy group is understood to mean a group containing 2 to 5 --OH groups.

Among the preferred ##STR13## groups, there may be mentioned those possessing 2 to 4 hydroxyl groups and containing 5 to 6 members, especially:

the groups ##STR14##

A first group of preferred compounds according to the invention is that having the following formula II: ##STR15## wherein R.sub.4 is --CH.sub.2 CH.sub.2 OH or --CH.sub.2 --CHOH--CH.sub.2 OH and R.sub.10 is --H or --CH.sub.3.

The compound of the following formula IIa is particularly preferred: ##STR16##

A second group of preferred compounds according to the invention is that having the following formula III: ##STR17## wherein R.sub.2 is ##STR18## R.sub.4 is as defined above for formula I, and R.sub.10 represents --H or --CH.sub.3.

The compounds of the following formula IIIa, IIIb, IIIc, IIId and IIIe are particularly preferred: ##STR19##

A third group of preferred compounds according to the invention is that having the following formula IV: ##STR20## wherein R.sub.2 is selected from the groups ##STR21## R.sub.5 is as defined above for the formula I and R.sub.10 represents --H or --CH.sub.3.

The compounds of the following formula IVa, IVb, IVc, IVd, IVe and IVf are particularly preferred: ##STR22##

A fourth group of preferred compounds according to the invention is that having the following formula V: ##STR23## wherein: R.sub.2 is --CONHCH.sub.3, ##STR24## R.sub.16 is as defined above for the formula I and R.sub.10 represents --H or --CH.sub.3.

The compounds of the following formulae Va, Vb, Vc, Vd and Ve are particularly preferred: ##STR25##

A fifth group of preferred compounds according to the invention is that having the following formula VI: ##STR26## wherein R.sub.4 is as defined above for the formula I, R.sub.9 represents --CH.sub.3 or --CH.sub.2 CH.sub.2 OH and R.sub.10 represents --H or --CH.sub.3. The compounds of the following formulae VIa and VIb are particularly preferred: ##STR27##

A sixth group of preferred compounds according to the invention is that having the formula VII below: ##STR28## wherein R.sub.9 and R.sub.10 are as defined for the formula I.

Among the compounds of the invention which contain a cyclic amine, another preferred product is: ##STR29##

Among the compounds of the invention of the dimeric type the products of formulae below are preferred: ##STR30##

The compounds of formula I in which the group R.sub.2 is different from the group --COR.sub.1 may be advantageously prepared by the process consisting of the following stages:

a) reduction, under usual conditions, of a compound of formula VIII ##STR31## wherein R.sub.2 represents the group ##STR32## and R.sub.8 are as defined above for the formula I, to give a compound of formula IX ##STR33## R.sub.2 being as defined above, b) iodination of the compound of formula IX to give a compound of formula X: ##STR34## wherein R.sub.2 is as defined above c) optionally, protection of the hydroxyl groups of R.sub.2 by means of a conventional protecting group; and,

d.sub.1) chlorination of the compound of formula X, under usual conditions, to give a compound of formula XI: ##STR35## wherein R'.sub.2 represents the group R.sub.2, optionally protected, e.sub.1) acylation of the compound of formula XI under usual conditions, by means of a compound of formula XII:

R'.sub.9 COCl (XII)

wherein R'.sub.9 represents R.sub.9 whose hydroxyl groups are protected, to give a compound of formula XIII: ##STR36## wherein R'.sub.9 and R'.sub.2 are as defined above, f.sub.1) amidation of the compound of formula XIII, under usual conditions, with an aminoalcohol selected from the compounds of formula: ##STR37## R.sub.4, R.sub.5, R.sub.6 and R.sub.16 being as defined above for the formula I, to give a compound of formula XIV: ##STR38## wherein R'.sub.2, R'.sub.9 and R.sub.1 are as defined above, g.sub.1) optionally, deprotection of the hydroxyl groups of R'.sub.2 and R'.sub.9, and

h.sub.1) optionally, alkylation, under usual conditions, of the compound of formula XIV with a compound of the formula XV:

R.sub.10 --X (XV)

R.sub.10 being as defined above and X being a movable group such as Br, Cl or I, to give a compound of formula I.

The compounds of formula I in which R.sub.2 =--COR.sub.1, R.sub.1 and R.sub.3 being as defined above for the formula I, can also be prepared in the following manner:

a) reduction, under usual conditions, of a compound of formula XVI: ##STR39## to give a compound of formula XVII: ##STR40## b) iodination of the compound of formula XVII to give the compound of formula XVIII: ##STR41## c) chlorination under usual conditions, of the compound of formula XVIII to give the compound of formula XIX: ##STR42## d) acylation of the compound of

formula XIX under usual conditions, by means of a compound of formula XII, as defined above, to give a compound of formula XX: ##STR43## R'.sub.9 being as defined above, e) amidation of the compound of formula XX, under usual conditions, with an aminoalcohol selected from ##STR44## R.sub.4, R.sub.5, R.sub.6 and R.sub.16 being as defined above for the formula I, so as to give a compound of formula XXI: ##STR45## in which R.sub.1 and R'.sub.9 are as defined above. f) optionally, deprotection of the hydroxyl groups of R'.sub.9

g) optionally, alkylation, under usual conditions, of the compound of formula XXI with a compound of formula XV as defined above, to give a compound of formula I.

The compound of formula VIII is obtained according to the method described in the patent FR-no. 6777M.

The reaction of stage a) is advantageously carried out by catalytic reduction by hydrogen over palladised carbon or over Raney nickel or by chemical reduction under usual conditions.

The iodination step is carried out under usual conditions such as by means of aqueous ICl or I.sub.2, in the presence of KI/ethylamine or KICl.sub.2, at temperatures ranging from 0.degree. C. to 100.degree. C.

The chlorination reactions are carried out in the usual manner, for example by means of SOCl.sub.2 or PCl.sub.5 at high temperature.

The acylation reactions are advantageously carried out in a solvent such as DMAC.

The amidation reactions are advantageously carried out in the presence of triethylamine.

The reactions for deprotecting the hydroxyl groups are advantageously carried out in the presence of K.sub.2 CO.sub.3 in methanol or NaOH, H.sub.2 SO.sub.4 or HCl.

The reaction for alkylating the compound of formula IX is advantageously carried out in the presence of NaOH, KOH, MeONa in DMAC, DMF or monoglyme.

The acetylation reaction is preferably carried out using acetic anhydride in a solvent, in the presence of pyridine, HClO.sub.4, H.sub.2 SO.sub.4, DMAP or using acetyl chloride at high temperature.

The compounds of formula VII are prepared by the process described above from 5-acetamido-2,4,6-triiodoisophthaloyl dichloride and the aminoalcohol of formula ##STR46##

The subject matter of the invention is also new aminoalcohols which are intermediate products in the preparation of test products for diagnosis. The aminoalcohols are especially useful as agents which enhance the biocompatibility of in particular iodinated contrast products, and more particularly the compounds of formula I.

A first group of new aminoalcohols have the following formula: ##STR47## in which R.sub.12 represents a group selected from --CH.sub.3, --CH.sub.2 CH.sub.2 OH, --CH.sub.2 --CHOH--CH.sub.2 OH, --CH--(CH.sub.2 OH).sub.2 and --CH.sub.2 (CHOH).sub.2 --CH.sub.2 OH.

A second group of new aminoalcohols have the following formula XXIII: ##STR48## in which R.sub.13 is selected from --CH.sub.3, --CH.sub.2 CH.sub.2 OH, --CH.sub.2 --CHOH--CH.sub.2 OH, and --CH(CH.sub.2 OH).sub.2.

A third group of new aminoalcohols have to the following formula XXIV: ##STR49## in which R.sub.14 is a group selected from --CH.sub.3, --CH.sub.2 CH.sub.2 OH and --CH.sub.2 CHOHCH.sub.2 OH.

The preparation of the following aminoalcohols will be described below:

aminoalcohol of formula XXII in which R.sub.12 represents --CH.sub.3 (compound no. 1),

aminoalcohol of formula XXII in which R.sub.12 represents the group --CH.sub.2 CHOH--CH.sub.2 OH (compound no. 2),

aminoalcohol of formula XXII in which R.sub.12 represents the group CH.sub.2 CHOH (compound no. 3),

aminoalcohol of formula XXII in which R.sub.12 represents --CH(CH.sub.2 OH).sub.2 (compound no. 4),

aminoalcohol of formula XXIII in which R.sub.13 represents ##STR50## aminoalcohol of formula XXIII in which R.sub.13 represents --CH.sub.3 (compound no. 6),

aminoalcohol of formula XXIII in which R.sub.13 represents --CH.sub.2 CH.sub.2 OH (compound no. 7),

aminoalcohol of formula XXIII in which R.sub.13 represents --CH(CH.sub.2 OH).sub.2 (compound no. 8),

aminoalcohol of formula XXIV in which R.sub.14 represents the group --CH.sub.3 (compound no. 9),

aminoalcohol of formula XXIV in which R.sub.14 represents --CH.sub.2 CH.sub.2 OH (compound no. 10),

aminoalcohol of general formula XXIV in which R.sub.14 represents the group --CH.sub.2 --(CHOH)--CH.sub.2 OH (compound no. 11),

aminoalcohol of formula XXII in which R.sub.12 represents --CH.sub.2 --(CHOH).sub.2 --CH.sub.2 OH (compound no. 12).

Preparation of the aminoalcohol no. 1 ##STR51## a) Preparation of the compound of formula ##STR52##

2 g (13.7 mmol) of 2,4-ethylidene-D-erythrose obtained according to the process described in J. Am. Chem. Soc. 2301, 1960, Barker R. et al., are dissolved in 10 cm.sup.3 of water at 30.degree. C. 10 cm.sup.3 of an aqueous solution of methylamine (40%) are added dropwise at 0.degree. C. After returning to room temperature, the stirring is continued for 2 h. The solution is then reduced, at room temperature, in the presence of palladium on carbon. The catalyst is then filtered and the filtrate concentrated to dryness. After solidification in ethyl ether, 1.7 g of the title product are obtained, equivalent to a yield of 77%.

TLC (dioxane/H.sub.2 O/NH.sub.3 :8/3/2) Rf: 0.74

TLC (CH.sub.2 Cl.sub.2 /MeOH 8/2) Rf: 0.17.

.sup.13 C NMR (DMSO) (.delta.,ppm) 200 MHz 98.2--(C--CH); 80.3 (CH--O); 70.5 (CH.sub.2 --O); 63.4 (CHOH); 53.1 (CH.sub.2 --N); 36.5 (NH--CH.sub.3); 20.7 (C--CH.sub.3).

b) Preparation of the compound of formula: ##STR53##

1.5 g (9.3 mmol) of the product obtained in a) are dissolved in 20 cm.sup.3 of 2N HCl. The solution is stirred at 50.degree. C. for 5 h. After concentration, and purification by passing through an H.sup.+ resin, the solution is evaporated to dryness. The residue is taken up in ethyl ether. After filtration and drying, 0.8 g of the title product is obtained (yield: 64%).

TLC (dioxane/H.sub.2 O/NH.sub.3 :8/3/2) Rf: 0.18

.sup.13 C NMR (DMSO) (.delta.,ppm):200 MHz 74.5--(CH--CH.sub.2 OH); 69.6 (CHOHCH.sub.2); 63.3 (--CH.sub.2 OH); 54.7 (--CH.sub.2); 36.12 (NHCH.sub.3) MS (DCI/NH.sub.3) m/z: 153 (M+N.sup.+ H.sub.4); 136 (M+H.sup.+) base peak.

Preparation of the aminoalcohol no. 2 ##STR54## a) Preparation of the compound of formula: ##STR55##

The compound is prepared according to the method described above.

The amino-reduction of 2,4-ethylidene-D-erythrose (6 g, 41 mmol) is carried out in the presence of aminopropanediol (1.2 equiv.) in ethanol (40 cm.sup.3).

After chromatography on a silica column, the title product is obtained with a yield of 73%.

TLC (dioxane/H.sub.2 O/NH.sub.3 :8/3/2) Rf: 0.73 .sup.13 C NMR (DMSO) (.delta.,ppm):(200 MHz) (98, C --CH.sub.3); (80.2-80.5, CH--O); (70.2-70.4 CH.sub.2 --O); (70.3.CHOH); (64.5-64.6, CH.sub.2 OH); (62.2-63.1, CHOH);(52.9-53, CH.sub.2); (50.8-51, CH.sub.2); (20.5, CH.sub.3).

b) Preparation of the compound of formula: ##STR56##

6 g (29.8 mmol) of the product obtained in the preceding stage are deprotected by treatment with 5N HCl (50cm.sup.3). The reaction medium is stirred for 4 h at 50.degree. C. After evaporation, the residue obtained is purified on an H.sup.+ resin. After concentration and solidification in ethyl ether, 2.6 g of the title product are obtained (yield 54.7%)

TLC (dioxane/H.sub.2 O/NH.sub.3 :8/3/2) Rf: 0.39

.sup.13 C NMR (DMSO) (.delta.,ppm) 74.3 (CH--CH.sub.2 OH butanetriol chain); 70.3 (CH--CH.sub.2).times.2; 64.5-64.6 (CH.sub.2 OH butanetriol chain); 63.3 (CH.sub.2 OH); 52.8 (CH.sub.2 N).times.2

MS (DCI/NH.sub.3) m/z 196 (M+H.sup.+) base peak;178 (M+H.sup.+ --H.sub.2 O); 160 (M+H.sup.+ --2H.sub.2 O) 136, 122, 109, 92.

Preparation of the aminoalcohol no. 3 ##STR57##

Just like methylamine (for the preparation of the aminoalcohol no. 1) and aminopropanediol (for the preparation of the aminoalcohol no. 2), ethanolamine, under the same amino-reduction conditions, gives, presence of 2,4-ethylidene-D-erythrose, the title product.

a) Preparation of the compound of formula ##STR58##

TLC(CH.sub.2 Cl.sub.2 /MeOH/NH.sub.3 :8/2/1) Rf: 0.56

.sup.13 C NMR (DMSO) (.delta.,ppm): 97.9 (C--CH.sub.3); 80.5 (CH--O); 70.2 (CH.sub.2 OH); 62.9 (CHOH); 60.2 (CH.sub.2 --O); 51.6(CH.sub.2 --N); 50.7 (CH.sub.2 --N); 20.4 (CH.sub.3).

b) Preparation of the compound of formula ##STR59##

TLC(CH.sub.2 Cl.sub.2 /MeOH/NH.sub.2 55/30/10) Rf: 0.25

TLC(dioxane/H.sub.2 O/NH.sub.3 :8/3/2) Rf: 0.48

.sup.13 C NMR (DMSO) (.delta.,ppm): 74.5 (CHOHCH.sub.2 OH); 70.2 (CHOH--CH.sub.2); 63.5 (CHOHCH.sub.2 OH); 60.4 (CH.sub.2 CCH.sub.2 OH); 52.5 (CH.sub.2 --CHOH); 51.8 (CH.sub.2 CH.sub.2 OH).

By repeating the procedures described above and by using serinol with 2,4-ethylidene-D-erythrose, the aminoalcohol of formula ##STR60## (aminoalcohol no. 4) is obtained. Preparation of the aminoalcohol no. 5 ##STR61##

3 g (18 mmol) of 2,3-epoxy-1,4-butyldiol prepared according to the method described in J. Med. Chem. 1976, vol. 19, No. 1, 153-158, are dissolved in 10 cm.sup.3 of methanol. 0.9 equiv. of aminopropanediol in 10 cm of methanol is added dropwise, at room temperature. The reaction medium is maintained at 45.degree.-50.degree. C. for 48 h. After evaporation, the crude product is purified with an H.sup.+ resin and concentrated to dryness. After taking up in ether and drying, 4 g of the title product are obtained (yield 72.7%).

TLC (dioxane/H.sub.2 O/NH.sub.3 :8/3/2) Rf: 0.58 (CH.sub.2 Cl.sub.2 /MeOH/NH.sub.3 : 6/3/1) Rf: 0.55

.sup.13 C NMR (DMSO) (.delta.,ppm): 71-71.2 (CHOH);64.6(--NH--CH--CH.sub.2 OH); 63.5 (CH.sub.2 OH propanediol chain); 61.4 (--CH--); 61 (CH.sub.2 OH); 51.3 (CH.sub.2 --N).

The opening of the epoxide described above can also be performed using methylamine, ethanolamine and serinol so as to obtain, respectively, the compounds: ##STR62## Preparation of the aminoalcohol of formula: ##STR63##

18.1 g (0.1 mol) of 3-bromomethyl-3-hydroxymethyloxetane, prepared according to the method described in Propellants, Explos., Pyrotech., 16(1)40-42, 1991, are stirred in 20 ml of methanol and 76 ml (1 mol) of 40% aqueous methylamine at 50.degree. C., for 24 h The mixture is evaporated to dryness and the residue is dissolved in 100 ml of 0.1N sulphuric acid.

The solution is refluxed for 12 h and then treated by a resin. The title product is obtained by evaporation of the eluent.

Preparation of the aminoalcohol of formula no. 10: ##STR64##

18.1 g (0.1 mol) of 3-bromomethyl-3-hydroxymethyloxetane, obtained as described above are stirred in 20 ml of methanol and 60.5 ml (1 mol) of ethanolamine at 50.degree. C., for 24 h. The mixture is evaporated to dryness and the residue is dissolved in 100 ml of 0.1N sulphuric acid. The solution is refluxed for 12 h and then treated by a resin. The title compound is obtained by evaporation of the eluent.

In the same manner as for the aminoalcohols nos. 9 and 10, the aminoalcohol no. 11 of formula: ##STR65## is obtained from 3-bromoethyl-3-hydroxymethyloxetane and 1-amino-2,3-propanediol.

Preparation of the aminoalcohol no. 12: ##STR66##

3,4-butanediol is prepared according to the rearrangement method described in patent U.S. Pat. No. 4,661,646 from 1,4-butanediol (available, commercially from the company Aldrich-Strasbourg).

Epoxidation of the 3,4-butanediol is carried out according to the method described in patent U.S. Pat.

No. 3,352,898 and gives 3,4-epoxy-1,2-butanediol.

The opening of the epoxy diol with benzylamine (0.5 equiv.) gives bis(2,3,4-trihydroxybutyl) benzylamine.

After debenzylation, by hydrogen, in the presence of palladised carbon, bis(2,3,4-trihydroxybutyl) amine(aminoalcohol no. 12) is obtained.

The aminoalcohol of formula ##STR67## is prepared as described in Tetrahedron Letters, 31, 6777 (1990), J. Org. Chem. 50, 892 (1985) or J. Chem. Soc. Chem. Commun. 262 (1987).

The aminoalcohol of formula XXIII, in which R.sub.13 represents H, is prepared as described in U.S. Pat. No. 4,341,756 and U.S. Pat. No. 4,439,613.

The aminoalcohol of formula XXV ##STR68## in which R.sub.15 represents --CH.sub.3 is prepared as described in J. Org. Khim., 22 (2), 298, 1986.

The aminoalcohol of formula XXV, in which R.sub.15 represents --CH.sub.2 CH.sub.2 OH, is marketed by Eastman Kodak.

The aminoalcohol of formula XXV, in which R.sub.15 represents ##STR69## is prepared as described in J. Am. Chem. Soc., 66 881, 1944.

The aminoalcohol of formula XXIV, in which R.sub.14 represents H, is prepared as described in Propellants, Explos. Pyrotech. 16(1), 40-42, 1991.

The aminoalcohols of general formula: ##STR70## are prepared in the following manner: R.sub.15 represents --CH.sub.3 (EP 25083);

R.sub.15 represents --CH.sub.2 CH.sub.2 OH (EP 25083, J. Med. Chem. 10(3), 511, 1967);

R.sub.15 represents ##STR71## (EP 25083).

The aminoalcohol of formula: ##STR72## is prepared as described in Angew. Chem. 6, 23, (1984), J. Med. Chem. Z, 1962 (1990).

The aminoalcohol of formula: ##STR73## is prepared as described in Tetrahedron Letters, 8, 857 (1984).

The aminoalcohol of formula: ##STR74## is prepared as described in J. Pharmacy and Pharmacology, 14 306 (1962).

The aminoalcohol of formula: ##STR75## is prepared as described in Chem. Ber., 8, 2467 (1967), Tetrahedron Letters, 15, 2139 (1990).

The aminoalcohol of formula: ##STR76## is prepared as described in G. Meng. M. Hesse, Helvetica Chimica Acta, vol. 74, p. 445-450 (1991).

Naturally, the invention encompasses not only the compounds of formula I in the form of racemic mixture. but also stereoisomers such as the enantiomers, diastereoisomers, optical isomers, and isomers SYN-ANTI, ENDO-EXO, E-Z, resulting from presence of asymmetric carbon atoms and/or limited rotation due to the steric hindrance caused by iodine atoms and/or by the substituents RH.sub.1, R.sub.2 and R.sub.3 of the compounds of formula I.

The subject matter of the present invention also the contrast media containing at least one compound

of formula I.

These contrast media are used in man and in animals for radiological purposes.

The preferred pharmaceutical forms of the contrast media according to the invention are aqueous solutions of the compounds.

The aqueous solutions generally contain a total of 5 to 100 g of at least one compound of formula I per 100 ml and the injectable quantity of such solutions may generally vary from 1 to 1000 ml.

The aqueous solutions of the compounds of formula I may also contain certain additives such as:

sodium chloride at concentrations of between 0.1 to 10 mM

EDTA disodium salt, at concentrations of between 0.1 and 2 mM

sodium citrate at concentrations of between 0.1 and 10 mM

heparin in amounts of between 10 and 100 units per 100 ml of solution,

and buffer solutions such as tris(hydroxymethyl)aminomethane hydrochloride.

These compositions may be administered by any route conventionally used for iodinated nonionic contrast agents. Thus, they can be administered enterally (orally, rectally) or parentally (intravenously, intraarterially, intraarticularly, opacification of the cavities), and in particular into the subarachnoid space, as well as by the bronchial, lymphatic and intrauterine routes.

In some special uses it may be necessary, in order to perform the diagnosis of a given pathology, especially in a specific organ, to have recourse to what is known as vectorisation of the contrast agent, which may be achieved by encapsulation of the said agent in liposomes or by its binding to a biomolecule, especially proteins.

An example of a composition according to the present invention will be given below.

Composition

Composition of example 2 65 g

Water for injection

qs 100 g

The following examples illustrate the preparation of the compounds of formula I

EXAMPLE 1

Preparation of 5-acetamido-N,N'dimethyl-N,N'-bis[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-2,4,6-triiodoisophthalamide of formula: (compound IVa) ##STR77## 1-Preparation of the compound of formula: ##STR78## 3-hydroxymethyl-3-methylaminomethyloxetane.

16 g (0.088 mol) of 3-bromomethyl-3-hydroxymethyloxetane, prepared according to M. A. Hiskey, Propellants, Explosives, Pyrotechnics, 16, 40-42 (1991), are stirred in 50 ml of ethanol with 69 ml (0.88 mol) of 40% aqueous methylamine, at 80.degree. C., under pressure, for 5 hours. The mixture is evaporated to dryness, then the residue is redissolved in 50 ml of ethanol, and 4.9 g (0.088 mol) of potassium hydroxide are added. After 1 hour, the suspension is filtered and the filtrate is evaporated

to dryness. The residue is taken up in 50 ml of dichloromethane. The suspension is filtered and then evaporated to dryness. The residue is distilled (b p: 160.degree. C., at 0.03 bars) and 10.2 g of 3-hydroxpethyl-3-methylaminomethyloxetane are obtained (yield 88.7%).

Analyses:

TLC: (SiO.sub.2): CH.sub.2 Cl.sub.2 --MeOH 8/2 Rf: 0.3

IR (KBr) 3380, 3300, 2940, 2870, 2800, 1450, 1070, 930 cm.sup.-1.

NMR:

.sup.1 H (DMSO-d.sub.6).delta.:4.27 (s, 4H, CH.sub.2 ring), 3.59 (s, 2H, CH.sub.2 --O), 2.71 (s, 2H, CH.sub.2 --N), 2.29 (s, 3H, CH.sub.3)

.sup.13 C (DMSO-d.sub.6).delta.:76.27; 64.32; 55.51 (methylene) 44.17 (quaternary), 37.06 (methyl).

2-Preparation of the compound of formula: ##STR79##

2,2-bis(hydroxymethyl)-3-methylamino-1-propanol(aminoalcohol no. 9)

10 g (0.08 mol) of 3-hydroxymethyl-3-methylaminomethyloxetane are dissolved in 30 ml of water with 6.5 ml of 18N sulphuric acid. The solution is refluxed for 16 hours and then cooled and eluted through anionic resin IRC 50. The eluate is evaporated to dryness and the residue is distilled

(b.p.: 180.degree.-190.degree. C., at 0.02 mbar) to give 6 g (yield: 50%) of 2,2-bis(hydroxymethyl)-3-methylaminopropanol.

Analyses:

TLC (SiO.sub.2) (methanol/ammonium hydroxide: 8/2)Rf: 0.39

I.R (KBr) 3290, 3250, 2960, 2940, 2880, 1450, 1020 cm.sup.-1

.sup.1 H (DMSO-d.sub.6).delta.4.10 (s, 3H, OH), 3.35 (s, 6H, CH.sub.2 O), 2.49 (s, 2H, CH.sub.2 N), 2.27 (s, 3H, CH.sub.3)

.sup.13 C (DMSO-d.sub.6).delta.62.77; 54.05 (methylene), 44.34 (quaternary), 37.32 (methyl).

3-Preparation of 5-acetamido-2,4,6-triiodoisophthaloyl dichloride

To a mixture of 16 ml of acetic anhydride (0.18 mol) and 2 ml of concentrated sulphuric acid are added 5 g of 5-amino-2,4,6-triiodoisophthaloyl chloride (0.008 mol) obtained according to the method described in FR 2, 343, 718. The stirring is continued for 1 hour at room temperature. 3.24 g of product are obtained after filtration, with a yield of 60.6%.

TLC (SiO.sub.2),(toluene-acetone: 80/20), Rf 0.37.

4-Preparation of 5-acetamido-N,N'-dimethyl-N,N'-bis-[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-2,4,6-triiodoisophthalamide

10 g (0.0156 mol) of 5-acetamido-2,4,6-triiodoisophthaloyl dichloride, obtained in the preceding stage, are dissolved in 30 ml of dimethylacetamide containing 6.5 ml (0.047 mol) of triethylamine.

6.9 g (0.047 mol) of 2,2-bis(hydroxymethyl)-3-methylamino-1-propanol, obtained in stage 2 above, are slowly added to the solution, at a temperature of 50.degree. C. The stirring is continued for 6 hours at this temperature, and then the solution is evaporated to dryness. The residue is taken up in water and is eluted through the resins IRN 77 and IRA 67. After evaporation to dryness, 9.5 g of a white powder are obtained (yield 70%).

TLC (SiO.sub.2)-butanol/H.sub.2 O/CH.sub.3 COOH 50/25/11

Rf: 0.49; 0.53

13CH.sub.2 Cl.sub.2 methanol 8/2

Rf: 0.82.

EXAMPLE 2

Preparation of the compound of formula: (compound Vc) ##STR80##

5-acetamido-N,N'-dimethyl-N,N'-bis(2,3,4-trihydroxybutyl)-2,4,6-triiodoisop hthalamide.

1.2 g (1.85 mmol) of 5-acetamido-2,4,6-triiodoisophthaloyl dichloride, as described in Example 1-3) above, is added to a solution of 1 g (7.4 mmol) of the aminoalcohol no. 1 prepared according to the method described above, and triethylamine (7.4 mmol) in 7 ml of dimethylacetamide. The stirring is continued for minutes at 40.degree. C. The triethylamine hydrochloride is removed by filtration.

The filtrate is evaporated to dryness, taken up in water and eluted through the resins IRN 77 and IRA 67. After evaporation to dryness, 1.32 g of a white powder are recovered (yield 84%).

Iodine assay: 99.5%

HPLC purity: 99% Lichrosphere C.sub.18 5 .mu.m 0.01M NaH.sub.2 PO.sub.4 MeOH

TLC (SiO.sub.2) CH.sub.2 Cl.sub.2 /MeOH: 70/30, Rf: 0.33, 0.26, 0.58 dioxane/water/NH.sub.3 : 80/30/20, Rf 0.74

.sup.1 H NMR (DMSO) 200 MHz (.delta.,ppm): (2.1, s, 3H, NHCOCH.sub.3); (2.85, s, 6H, N--CH.sub.3); (3,1-4, broad 12H, CH and CH.sub.2); (4.3-4.9, un-broad 6H, OH) (10, broad 1H, NH)

.sup.13 C (.delta.,ppm): (22.28, NHCOCH.sub.3); (37.76, N--CH.sub.3); (50.58, N--CH.sub.2); (62.35, CH.sub.2 OH); (69.89-73.4, CHOH); (89.45-97.4-98.8, 2C--I) (143.69, C.sub.ar --N); (148-148.6. C.sub.ar --CO) (171.9-172.1, C.dbd.O).

EXAMPLE 3

Preparation of the compound of formula: (compound Vb) ##STR81##

[N-(2,3-dihydroxypropyl)-N-(2,3,4-trihydroxybutyl)]-3-(N'-methyl)-5-N-methy lacetamido-2,4,6-triiodoisophthalamide.

1 g (1.54 mmol) of 5-N-methylacetamido-2,4,6-triiodo-N-methylbenzamide chloride prepared according to the method described in FR 2,272,640, is added to a solution of 542 mg (3.09 mmol) of the aminoalcohol no. 2 obtained according to the method described above, and triethylamine (3.09 mmol) in 7 ml of dimethylacetamide. The stirring is continued for 4 hours at 60.degree. C.

The solution is then concentrated by distillation. After purification with the resins IRN 77 and IRA

67 and evaporation to dryness, 1 g of a white powder is obtained (yield: 82%).

TLC (SiO.sub.2)CH.sub.2 Cl.sub.2 /MeOH: 7/3: Rf 0.58 dioxane/H.sub.2 O/NH.sub.3 : 80/30/20 Rf: 0.89

.sup.13 C NMR 200 MHz (.delta.,ppm): (24.22, N--CH.sub.3); (25.73, COCH.sub.3); 33.65, NHCH.sub.3); (49.63-53.54, N(CH.sub.2).sub.2); (61.8-63.3, CH.sub.2 OH); (68.64-73.28, CHOH); (90,56-98,01, C--I); (147.9-148.1, C--CO); (150.33, C.sub.ar --N); (171.6-172.9, C.dbd.O).

EXAMPLE 4

Preparation of the compound of formula: (compound IIa) ##STR82##

5-acetamido-N,N'-bis(2-hydroxymethyl)-N,N'-bis[1-hydroxymethyl)-(2-hydroxy) ethyl]-2,4,6-triiodoisophthalamide

To 4.17 g (6.94 mmol) of 5-acetamido-2,4,6-triiodoisophthaloyl dichloride, obtained according to the method described in Example 1-3 above, is slowly added a solution of 3.53 g (0.026 mmol) of the aminoalcohol ##STR83## obtained according to the process described in EP 25083 and J. Med. Chem. 10/3, 511, 1967, and 2.63 g of triethylamine in 20 ml of dimethylacetamide.

The stirring is continued for 48 hours at 50.degree. C. The excess triethylamine and N,N-dimethylacetamide is removed by distillation. The residue is taken up in water and passed through H.sup.+ and OH.sup.- ion-exchange resins. After evaporation and crystallisation, 3.5 g of product are obtained (yield: 64%).

TLC (SiO.sub.2):dioxane/H.sub.2 O/NH.sub.3 : 80/30/20, Rf: 0.8

HPLC purity: 99% Lichrosphere C.sub.18 5 .mu.m Buffer MeOH

.sup.13 C NMR (DMSO) (.delta.,ppm):

(23.02, COCH.sub.3); (50, --N--CH.sub.2 --CH.sub.2 OH) ;(59.30, CH(CH.sub.2 OH).sub.2); (62 C H(CH.sub.2 HO).sub.2); (92-100 3C--I); (145-148 C.sub.ar --N and C.sub.ar --CO); (167-170, C.dbd.O).

EXAMPLE 5

Preparation of the compound of formula: (compound VIb) ##STR84##

1 g (1.54 mmol) of 5-N-methylaceteido-2,4,6-triiodo-3-N-methylaminocarbonylbenzamide chloride, prepared according to the method described in FR-2,272,640, is added to a solution of 542 mg (3.09 mmol) of the einoalcohol no. 5 obtained according to the method described above, and triethylamine (3.09 mmol) in 5 ml of dimethylaceteide. The stirring is continued for 48 h at 65.degree. C.

The solution is concentrated by distillation. After purification through the resins H.sup.+ IRN 77 and OH.sup.- IRA 67, and evaporation to dryness, the title product is obtained in the form of a white powder.

TLC (SiO.sub.2) CH.sub.2 Cl.sub.2 --MeOH 7/3, Rf: 0.49 dioxane/H.sub.2 O/NH.sub.3 80/30/20, Rf: 0.8.

EXAMPLE 6

Preparation of the compound of formula: (compound Ve) ##STR85##

5-acetamido-3-(N'-2-hydroxyethyl)-N-(2-hydroxyethyl-2',3',4'-trihydroxybuty l)-2,4,6-triiodoisophthalamide

To 675 ml (7.15 mol) of acetic anhydride, heated to 50.degree. C., are slowly added 250 g (0.42 mol) of 3-(N-hydroxyethylcarbamoyl)-5-amino-2,4,6-triiodobenzoic acid, obtained according to the method described in patent FR 6777.M.

The temperature is maintained around 55.degree. C. during the addition of 1.5 ml of perchloric acid.

The stirring is continued for 8 hours at 50.degree. C. After distillation under reduced pressure, 380 ml of butyl acetate are added after cooling. The reaction medium is neutralised by adding 1.5 g of sodium acetate and stirring is continued for 12 hours at room temperature. The medium is then heated to 70.degree. C. and 168 ml (2.34 mol) of thionyl chloride are slowly added while the heating is maintained for 2 hours. The thionyl chloride and the acetyl chloride generated are then removed by distillation under reduced pressure.

The residue is taken up in 150 ml of butyl acetate, and then stirred for 3 hours at 10.degree. C. The product is filtered, rinsed with butyl acetate and drained.

Yield: 78%

TLC (toluene/methyl ethyl ketone/formic acid: 65/35/25) Rf: 0.7

.sup.1 H NMR ((DMSO-d.sub.6):2 ppm (s, 33H);2.15 (s, 3H); 2.35 (s, 3H) 3.5 (b, 2H); 4.15 (b, 2H); 9.8 (b, 1H).

100 g (133.9 mmol) of 5-N,N-diacetylamino-3-(N'-2-acetoxyethyl)carbamoyl-2,4,6-triodobenzoic acid chloride, obtained in the preceding stage, are added to a solution of 44.2 g (268 mmol) of the aminoalcohol no. 3 obtained according to the method described above, and triethylamine (268 mmol) in 220 cm.sup.3 of dimethylacetamide. The stirring is continued for 2 hours at 55.degree. C. Triethylamine hydrochloride is removed by filtration and the filtrate is evaporated to dryness. The residue is diluted in 500m 1 of water and contacted with 2N NaOH (180 ml) for 12 hours. The solution is then neutralised and eluted through resins IRN 77 and IRA 67.

After evaporation to dryness, 58.4 g of product are obtained, equivalent to a yield of 55%.

TLC: (SiO.sub.2) CH.sub.2 Cl.sub.2 /MeOH/NH.sub.3 : 8/3/2 Rf: 0.27+0.41

Iodine assay: 98.4%

HPLC purity: 99.5% (Raw) 95%

Lichrosphere C.sub.18 5 .mu.m; NaH.sub.2 PO.sub.4 0,01M; MeOH

.sup.1 H NMR (DMSO) 200 MHz (.delta.,ppm): (2.01, S, 3H, NHCOCH.sub.3); (3.85-4.15; broad 14H, CH and CH.sub.2 ; (4.3-5.1 broad 5H, OH); (8.5, broad 1H, ArCONH); (9.9, broad 1H, ArNHCO).

EXAMPLE 7

Preparation of the compound of formula: ##STR86##

1,3-bis[(2S,5S)-dihydroxymethyl)-(3R,4R)-dihydroxy-pyrrolidin-1-yl-carbonyl]-5-acetamido-2,4,6-triiodophenyl.

28.45 G (0.1745 mol) of (2S,5S)-dihydroxymethyl-(3R,4R)-dihydroxypyrrolidine are stirred in the presence of 24.5 cm.sup.3 of triethylamine and 200 cm.sup.3 of DMAC at 50.degree. C. 31.82 g (0.05 mol) of 5-acetamido-2,4,6-triiodoisophthaloyl chloride, as prepared in Example 1-3 above, are slowly added and the reaction is maintained at 50.degree. C. for 48 hours. The DMAC is then evaporated and the reaction medium is taken up in dichloromethane, filtered and then evaporated. The oil is then diluted to a volume of 200 cm.sup.3 and then passed through H.sup.+ and OH.sup.- ion-exchange resins. The aqueous phase is evaporated to dryness. The product is obtained, after drying in an oven and purification by preparative HPLC, with a yield of 23%.

TLC (CHCl.sub.3 55/MeOH45/NH.sub.4 OH10): Rf=0.14; 0.2

Iodine purity: 100.1%

H.sub.2 O content 2%

HPLC purity: 99%

.sup.1 H NMR (DMSO): .delta.: 10.0 (b. 1H, NH), 5.5 (b 8H, OH) 4 to 2.8 (b. 16H, CH.sub.2 --CH) 2.0 (1. 3H, CH.sub.3)

.sup.13 C NMR (D.sub.2 O+C.sub.6 H.sub.6 standard): .delta.: 172 (C.dbd.O, NHCO), 170.0 (C.dbd.O, CO--N); 147.6-147.1-143.1 (C9); 101.1-98,8-91.3 (CI); 72.7-72.5 (CHOH); 59.8-59.3 (CH--N); 58.4-56.9 (CH.sub.2 --OH); 21.7 (CH.sub.3).

EXAMPLE 8

Preparation of the compound of formula: ##STR87##

A mixture of 36.50 g (0.27 mol) of N-methylaminobutanetriol (aminoalcohol no. 1), 27.27 g (0.27 mol) of triethylamine and 400 ml of isopropanol are heated to 40.degree. C.

TO this mixture are added 71.70 g (0.057 mol) of bis[3,5-bis(chlorocarbonyl)-2,4,6-triodophenyl] malonamide, prepared according to the method described in patent U.S. Pat. No. 4,426,371 in the name of SCHERING AG, dissolved in 415 ml of isopropanol. The solution is maintained at 40.degree. C. for 4 hours and left at room temperature overnight. The reaction medium is filtered. The precipitate is passed through H.sup.+ and OH.sup.- resins, taken up in absolute ethanol and then in water, evaporated to dryness and dried (yield 63%).

Analysis: TLC (SiO.sub.2) CH.sub.2 Cl.sub.2 -MeOH (4/6) visualisation: UV

Rf: 0.27

Iodine purity: 99.43% .sup.1 H NMR (DMSO).delta.: 12.0 (b, 2H, NH), 4.6 to 4.4 (b,12H, OH), 3.8 (b, 4H, CHOH), 3.5 (b, 8H, CH.sub.2 OH), 3.4 (b 2H, CH.sub.2, 3.2 (b, 8H, NCH.sub.2), 3.1 to 2.6 (b, 12H, CH.sub.3);

.sup.13 C (D.sub.2 O+C.sub.6 H.sub.6 standard .delta.: 171 (C.dbd.O, N--C.dbd.O), 166 (C.dbd.O, N--C.dbd.O), 148-147 (C cycl., C--C.dbd.O), 142 (C cycl., C--NH), 98 to 86 (C cycl., CI), 72 to 69 (CH, CHOH), 61 (CH.sub.2, CH.sub.2 OH), 50 (CH.sub.2, N--CH.sub.2), 37 to 33 (CH.sub.3, N--CH.sub.3)

MS (FAB): correct; M+1=1655

EXAMPLE 9

Preparation of the compound of formula ##STR88##

A mixture of 1.01 g (6.13 mmol) of (2S,5S)-bishydroxymethyl-(3R,4R)-bishydroxypyrrolidine prepared according to the method described in Tetrahedron Letters, 31, 6777 (1990), J. Org. Chem. 50, 892 (1985), of 0.62 g (6.13 mmol) of triethylamine and of 3 ml of N,N-dimethylacetaide is heated to 50.degree. C. To this mixture is added 0.88 g (0.70 mmol) of bis[3,5-bis(-chlorocarbonyl)-2,4,6-triiodophenyl]malonamide, prepared according to the method described in U.S. Pat. No. 4,426,371, dissolved in 2.5 ml of N,N-dimethylacetaide. The solution is maintained at 50.degree. C. for 24 hours and left at room temperature overnight. The reaction medium is filtered. The filtrate is passed through resins H.sup.+ and OH.sup.-, taken up in absolute alcohol then in water and evaporated to dryness and dried. 380 mg of a white powder are isolated.

Analysis

HPLC (70 CH.sub.3 OH/30 H.sub.2 O) t.sub.R =1.62 column C18.

EXAMPLE 10

Preparation the compound of formula: (compound Vd): ##STR89##

5-acetamido-3-(N-2-hydroxyethyl)-1-N'-(2,3,4-trihydroxybutyl-2', 3',-dihydroxypropyl)-2,4,6-triiodoisophthalamide:

100 g (133.9 mmol) of 5-N,N-diacetylamino-3-N'-(2-acetoxyethyl)carbamoyl-2,4,6-triiodobenzoic acid chloride, prepared according to the method described in Example 6 above, are added to a solution of 39.2 g (200 mmol) of the aminoalcohol no. 2 obtained according to the method described above, and triethylamine (267.8 mmol) in 220 ml of dimethylacetamide. The stirring is continued for two hours at 55.degree. C. The triethylamine hydrochloride is removed by filtration. The filtrate is evaporated to dryness. The residue obtained, diluted in 500 ml of water, is contacted with 2N sodium hydroxide (120 ml) for 12 hours.

The medium is then neutralised before being successively passed through the resins IRN 77 and IRA 67. After evaporation to dryness, 58.5 g of the compound Vd are obtained, equivalent to a yield of 53%.

TLC (SiO.sub.2)CH.sub.2 Cl.sub.2 /MeOH / NH.sub.3 : 7/3/2 Rf: 0.28

Iodine assay: 99.2%

Purity HPLC: (Lichrosphere C.sub.18 5 .mu.m NaH.sub.2 PO.sub.4 : 0.01M, MeOH): 99%

.sup.1 H NMR (DMSO) 200 MHz (.delta.ppm). (2.01; .delta.; 3H ; NHCOCH.sub.3); (3-4.2 ; broad 15H; CH and CH.sub.2); (8.5; broad, 1H, ArCONH); (9.9: broad, 1H, ArNHCO).

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